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ANNUAL REPORT of program activities

July 1, 1975 through June 30, 1976

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OFFICE OF THE DIRECTOR

OFFICE OF THE DIRECTOR
Summary Statement

During the first decade of the National Institute of Environmental Health Sciences existence, we have concentrated on three principal tasks:

--Conducting and supporting research aimed at the development of understanding of the factors in man's environment which have an adverse effect upon his health and the mechanisms through which that toxicity is expressed;

--Making the results of that research available to the medical profession, regulatory agencies, industry, and the general public;

--Serving as a consultant to various agencies and individuals regarding the potential health effect of proposed environmental activities.

The thrust of our activities during this period has been directed toward addressing those needs in the environmental health area we believe to be most important.

Intrinsic to our modern civilization are a great many forces and developments which are constantly increasing the need for research efforts in environmental health. Paramount among them is the development since World War II of a sophisticated, complex chemical process industry.

Every year there are between 500 and 1000 new chemicals introduced in commercial quantities. These compounds can affect society in a surprising variety of ways. There is, first of all, exposure of workers in the occupational environment where these compounds are created, processed, and packaged. Effluents from manufacturing plants can lead to pollution of the air or the water. Transport of these materials to processing plants can lead to pollution of water, air, or land areas adjacent to the transportation routes. And sometimes the final product or its disposal may have hazardous aspects. The problems we face in this area can often be totally unexpected.

A second major factor increasing the need for research efforts in environmental health is our constantly increasing use of energy. There are a great number of problems implicit in our growing demand for all forms of energy. Critical problems include the sulfur oxides and particulates in the air, the nitrogen oxides from the power plants and automobiles, and the hydrocarbons--which can cause cancer and genetic damage--that come from incomplete combustion, from coal liquification or gasification, or even from oil spills in the ocean. Another problem--which may be accentuated by the projected increase of coal utilization--is the discharge into the atmosphere of a variety of heavy metals of known and serious toxicity.

A third factor, perhaps more subtle than the others, is the constantly increasing urbanization of modern civilization. As our society has moved from a predominantly agrarian and cottage industry economy with small towns and widely dispersed populations to the intense development of our major metropolitan areas, we have found that the higher volumes of effluents from industrial and commercial operations, from transportation, and from residences result in the exposure of larger numbers of people. These problems would be less critical were the industries and the population distributed more evenly over the countryside.

Although we all enjoy the fruits of modern civilization and the developing technology which has allowed us to increase our standards of living to heights scarcely believable three decades ago, we must face the problems arising from the growing uncontrolled concentrations of toxic pollutants in all sectors of our environment.

We believe that research can accomplish the goal of preventing the disease, disability, and death caused by the proliferation of toxic environmental agents. We believe these goals will be accomplished either directly through aiding restriction of exposure or indirectly through understanding and amelioration. Although now and for the future the direct route will be important and often necessary, the indirect route will assume increasing importance.

The proscription or restriction of exposure can cause serious economic disruption. Certain toxic agents yield very real benefits. Further, large numbers of compounds to which man is now exposed have never been adequately tested for toxicity; and to do so with current methods would require too much time and too much money. If, however, we develop understanding of how agents exert their toxic effects, then we will be able to predict which agents may cause toxic effects and to test these intensively. We will then be able to prevent exposure to those agents which have the potential to cause irreparable harm. In this way, we can more effectively protect the public health.

The year just past brought a series of developments which indicate widespread recognition of the importance of environmental health problems in general and the programs of the National Institute of Environmental Health Sciences in particular. The effect of these developments is that at last we have begun to achieve a critical mass and should in the coming years make major contributions to the protection of the public health.

In 1977, for the first time since 1968, a budget was proposed for the NIEHS which provided an adequate increment for program development. With the exception of the continuing critical problem of inadequate numbers of staff, we believe that the Institute has "turned the corner" in terms of its development.

In its action on the 1976 Appropriation, the Congress provided $25 million to enable us to begin construction of our permanent facility. This development means that the Institute can look forward to occupying permanent quarters sometime during FY 1980. This means that we can begin the long delayed full development of our intramural program.

As a further significant development, we have underway a major planning activity aimed at the identification of research needs in environmental health. This study, which is being conducted primarily by non-government scientists, is to be completed by January, 1977. It should form the basis for our future program planning.

The Institute continues its strong commitment to its environmental health centers as an effective means of attacking major environmental health problems in a variety of areas such as heavy metals toxicity, community and occupational settings, air pollution, environmental carcinogenesis, and trace environmental contaminants.

Within the limits of available resources we are attempting to maintain a broad-based attack on a variety of environmental health problems.

With the additional resources made available in both the 1976 and 1977 budgets, we are proceeding with the further development of our environmental mutagenesis programs, our marine biomedical programs and behavioral toxicology as well as target organ toxicity.

OFFICE OF EXTRAMURAL PROGRAMS

SUMMARY STATEMENT

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS

Research Grants

During FY-1976, 249 individual research and program project grant applications were reviewed, an increase of 16 percent over FY-1975. Awards were made for 152 noncompeting and 15 competing projects from FY-1976 appropriations. In addition, 17 competing projects were supported from energy related pass through funds provided under an interagency agreement with the Environmental Protection Agency. Thus, regular research awards totaled 184, a decrease of 9 percent from FY-1975, in the amount of $14.9 million ($1.3 million energy funds included), an increase of 15 percent. Additionally, 10 applications for Research Career Development Awards were reviewed. Eight noncompeting and one competing were awarded in the amount of $212 thousand.

Training Programs

Ten institutional training grants awarded prior to passage of Public Law 93-348 were continued for a total of $856 thousand. Phaseout of these grants is continuing in accordance with provisions of the law. Seven Institutional National Research Service Awards initiated in FY-1975 were continued at a level of $856 thousand. Nineteen individual fellowships received noncompeting awards at a level of $292 thousand. No new institutional or individual awards were made during this fiscal year.

National Research Service Awards

On July 12, 1974, Public Law 93-348 was enacted providing authority for NIH and ADAMHA to make National Research Service Awards (NRSA's) for predoctoral and postdoctoral training. This program superceded all previous training programs but provided for orderly phaseout of existing programs. Thus, the NIEHS currently has three separate training programs--the "old" training grant and the "Weinberger" fellowship programs, which are being phased out, and the NRSA's, which were awarded for the first time from FY-1975 funds. On June 30, 1975, the authority for this program expired.

On April 22, 1976, the President signed into law the Health Research and Health Service Amendments of 1976 which extended NIH authority to make Individual and Institutional National Research Service Awards in support of both predoctoral and postdoctoral training. The new law included some modifications to the prior law of which the following are most significant: (1) authorizes awards for biomedical and behavioral research training at all public, including Federal, institutions; (2) broadens service payback alternatives to include not only health research or teaching but also any combination which is in accordance with usual patterns of academic employment; (3) provides for change in method of determining financial payback requirements in the absence of service payback; (4) requires that National Academy of Sciences annual studies regarding biomedical and behavioral research personnel

be conducted in consultation with the Director, NIH, and establishes September 30 of each year as the date for submission of such studies to the congress; and (5) authorizes appropriations for National Research Service Awards of $165 million in FY-1976, and $185 million in FY-1977. In the latter regard, funds for new starts during FY-1976 were dependent upon a supplemental appropriation. Action on such an appropriation was incomplete at the time of this annual report.

University-based Centers

University-based Centers are an integral part of the strategy of NIEHS in serving the national needs in environmental health research. They evolve as a consequence of the demonstrated ability of scientists within the same institution to perform high quality, multifaceted research in areas of high relevance to the mission of NIEHS and to attract and train outstanding students to meet future needs of the Nation. During FY-1976, the NIEHS supported seven Centers with funds totaling $5.3 million. Of these seven, one was a competing renewal.

Communication between the Centers and this Institute is maintained at an effective level through biannual meetings of the Center Directors, frequent visits and telephone contacts by Extramural Staff and through the National Advisory Environmental Health Sciences Council's Subcommittee for University-based Centers. The Subcommittee provides liaison between the Institute, the Advisory Council, and the Center Directors. It visits each Center in turn to discuss research problems and accomplishments, new environmental concerns, and program directions.

The 12th semi-annual meeting of the Center Directors was held on May 11-13, 1975, at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, with Dr. David P. Rall, Director, as host. This meeting was unique in that all 14 program project directors were invited and each director made a presentation on the highlights of the research being conducted within the program project. These presentations were followed with the regular program of presenting highlights by the Center Directors. Both the program project directors and the Center Directors felt that this was a worthwhile session and voted to continue the joint meetings on a periodic basis, probably every second time the group meets at NIEHS in order that the Directors of these two major facets of the NIEHS program may be aware of what research is being conducted within the program projects, the Centers, and the NIEHS Intramural program. The Directors of both types of programs also voted to exchange progress reports in the future to facilitate this integration of information.

The 13th annual meeting of the Directors of the University-based Centers was held at the Mount Sinai School of Medicine, New York, on October 5-7, 1975, with Dr. Irving J. Selikoff as host. The Sunday evening session was highlighted by a panel discussion lead by Dr. Rall in which various invited members of the news and television media and scientists outside of the NIEHS group discussed access to results of environmental research; integration of

timeliness, accuracy, and evaluation. Dr. Rall served as moderator and the panel members were Gershon Fishbein, Environmental Health Letter, Washington, DC; V. K. Rowe, Dow Chemical Company, Midland, Michigan; Ms. Judy Randal, National Association of Science Writers, Washington, DC; Earl Ubell, NBC-TV, New York City; and Sidney Wolfe, Health Research Group, Washington, DC. The open discussion after the panel members presentations was lively and informative and evoked so much favorable comment that a similar format was used at the March, 1976 meeting of the National Advisory Environmental Health Sciences Council. On Monday, Dr. Selikoff and his staff presented an in depth review of the research being supported under the Mount Sinai Center grant. On Tuesday, Dr. Rall presented NIEHS highlights of the Intramural Program, and the other Center Directors presented highlights of their programs during the previous year.

The University-based Center Subcommittee of the Council met prior to the November 1975 Council meeting to discuss the development of a set of guidelines for NIEHS University-based Centers. From this evening meeting evolved an outline which the Subcommittee and NIEHS staff will pursue, including involvement with the Center Directors toward the development of a more definitive plan of action and to maintain consistency in the application of the core center concept. The Subcommittee again met on the eve of the March 1976 Council meeting to review progress on the guidelines. It was decided to make a presentation during the formative state of development at the next Center Directors' Meeting to be held in May at Vanderbilt University. During this meeting the Subcommittee also discussed in depth their findings during a site visit review of the NIEHS University-based Center at Harvard, which was conducted in late January. The Subcommittee members attend all Center Directors' meetings and it is their goal to identify and correct any weaknesses in the program through joint discussions.

Supported Research Highlights

During the year, grantees made substantive contributions to each of the categorical programs of the Institute. The etiological relations of bioactive environmental agents, some new and some with long-standing usage, in human health were illuminated further. Progress was made in understanding the basic toxicological and pathological events in pulmonary and cardiovascular diseases, reproductive and genetic anomalies, neurological disorders and various other syndromes ascribable to environmental factors. Parallel advances were made in analytical methodology for specific toxicants and noxious dusts and in development of quantitative test systems for carcinogenesis and mutagenesis. Highlights from a number of programs which illustrate these achievements are presented herewith.

RESEARCH HIGHLIGHTS

Introduction

Because most questions about grant supported research are asked in terms of bioactive chemicals and physical agents instead of specific diseases, the following account of extramural research is presented in these terms. Broadly, the categories are:

- o Gaseous air pollutants
- o Suspended particulates (aerosols)
- o Industrial byproducts and intermediates
- o Heavy metals
- o Pesticides
- o Food additives and adulterants
- o Mutagens and carcinogens
- o Physical factors

Obviously, these are perceptual rather than conceptual descriptors, but they simplify review of the research somewhat and provide better focus on the nature and origin of environmental factors of concern to the NIEHS.

Gaseous Air Pollutants

Nitrogen Dioxide and Ozone

Etiology and pathogenesis. A number of NIEHS-supported investigators are making important contributions toward understanding the etiology, pathogenesis and other relations of NO_2 and O_3 to pulmonary and extrapulmonary disorders. Dr. Gustave Freeman et al. (Stanford Research Institute) have shown that rats exposed intermittently for a lifetime (about 2 1/2 years) to 15 ppm NO_2 develop a chronic obstructive lung disease with features much like those of human emphysema. O_3 was shown to be approximately 20 times more injurious than NO_2. The disease it induces is similar to that caused by NO_2, but the initial injury to tissues is slightly deeper in the lungs and there is an early stimulation of connective tissue growth at the sites of injury. A significant aspect of the studies is the fact that cellular changes are obvious within four hours of exposure to about 0.9 ppm O_3, a level occasionally reached in heavy smog. Mixtures of O_3 and NO_2 as low as 0.25 ppm and 2.5 ppm, respectively, caused definitive injury to the lining cells of the small airways of the lung within 24 hours. Mixtures equivalent to levels occasionally reached in smog (0.9 ppm O_3) induced an advanced lethal degree of emphysema in the test animals within two months upon continuous exposure.

The pathological events in the disease have been substantially clarified. Dr. Donald Dungworth (University of California, Davis) has shown that exposure to as little as 0.2 ppm O_3 for seven days causes mild but unequivocal damage in and adjacent to the smallest terminal airways. Dead cells and debris accumulate in these areas, hydrolytic enzymes are released, and changes in lining cells are easily seen by light or electron microscopy. Accumulation

of cellular debris results from sloughing of ciliated cells and of the thin, flat Type 1 cells from the alveolar walls.

Insight into the molecular bases of cellular injury has been provided by a number of investigators. Dr. Daniel Menzel (Duke University) and Dr. Orville Privette (University of Wisconsin) have shown that ozone exposure results in ozonolysis of polyunsaturated fatty acids. Much of the gross pathology caused by ozone inhalation was duplicated by injection of methyl ozonides of fatty acids. These compounds were found to initiate peroxidation in isolated organelles, which supports the hypothesis that O_3 and NO_2 induce membrane and cellular degeneration through lipid peroxidation. This hypothesis is strengthened further by the finding of Dr. Bernard Goldstein (New York University Medical Center). He has shown that on exposure of membranes to ozone, free radicals, peroxides and carbonyl compounds are formed from unsaturated lipids.

Other potentially important changes occur as sequalae to lipid peroxidation and membrane changes. Prostaglandins are released as they are in most cases of tissue damage (Dr. Privette). Age-related pigments (lipofuscins) are induced (Dr. Saari Csallany, University of Illinois). Pulmonary tissue oxygen consumption is increased, with an attendant increase of about 30 percent in a number of enzymes associated with metabolic regulation and in lytic enzymes capable of breaking down lung tissues (Dr. Dungworth). Cytosol thiols are oxidized and certain soluble proteins are polymerized (Dr. Menzel). These kinds of changes are in accord with the progression of tissue degeneration to the pathological condition seen in chronic obstructive lung diseases.

Using a new system for evaluation of sensory irritation in Swiss mice. Dr. Yves Alarie (University of Pittsburgh) has tested a series of hydrocarbon-nitrogen dioxide mixtures following u.v. irradiation to evaluate sensory irritation due to the products formed during irradiation. The hydrocarbons used were 1,3-butadiene, isobutylene, propylene, ethylene, butane, and cis-2-butane. In each case, a 3/1 ratio of hydrocarbon-nitrogen dioxide was used in an irradiation chamber and the mixture was evaluated for its potency as a sensory irritant. A rating of sensory irritation was devised and a correlation was found between the animal response and the human response. In order to "calibrate" the response in animals, acrolein was used as a sensory irritant and a dose-response curve was obtained. Acrolein was selected since it is one of the suspected products involved when unsaturated hydrocarbons-nitrogen dioxide mixture is used and is a chemical on which human data are available. Using the most potent combination found in animal systems and in humans (1,3-butadiene-nitrogen dioxide), four hour exposures revealed that in contrast to sulfur dioxide, which produced both irritation and desensitization in Swiss mice, the irritation level obtained with this mixture remained for the entire four-hour exposure and increased with repeated exposures.

In addition to effects directly on lung tissue, a number of studies have shown that O_3 and NO_2 produce other kinds of effects as well. Dr. Jean Acton (Bowman Gray School of Medicine) has demonstrated that macrophages are damaged. Using lactic dehydrogenase as an indicator of leakage from the cells, she has shown that exposure to 25 ppm NO_2 for as little as an hour results in

membrane damage to the cells. Concomitantly, Dr. Paul Southern (University of Texas) has found that NO_2 reduces the *in vivo* bactericidal capacity of alveolar macrophages. Thus, in addition to direct damage to lung tissues, individuals exposed to the gases may become prone to pulmonary infections.

Dr. Southern has tested effects of NO_2 and CO on mucociliary function and alveolar macrophage activity in rats, using a number of bacterial species. At levels of exposure that might be encountered in the environment, the data suggest that NO_2 effects primarily the activity of macrophages, with little or no effect on mucociliary function. As yet, no significant changes have been found with CO alone at low levels of exposure.

Fetal responses to NO_2 and O_3. Dr. Robert Stephens has initiated a relatively new program on comparative responses of fetal and postnatal lungs of rats to O_3 and NO_2. For current experiments, the proliferative "nodule" which develops in the lung at the alveolar duct after 48 hours of continuous exposure was chosen as a convenient point of reference. Such nodules, recognizable in rats under the dissecting microscope, are comparable when animals older than 35 days are exposed to either 0.9 ppm O_3 or 15 ppm NO_2. Although structural and cytodynamic changes are evident at levels of 0.25 ppm O_3 and 2 ppm NO_2, the response is more consistent from animal to animal at the higher levels.

Current research pertains to rats between 1 and 35 days old exposed to NO_2 or O_3 at the levels specified above. Weaning usually was on the twentieth day after birth, but, in various experiments, animals were weaned at 15 and at 25 and 30 days after birth.

The reaction of lung tissue of animals weaned at 20 days of age and exposed for 48 hours to 0.9 ppm O_3 at 35 days of age is dramatic. The lungs have prominent focal proliferative nodules at the level of the proximal end of the alveolar duct and terminal bronchiole. When this response is compared with that of younger animals between 1 and 24 days old, the neonate shows very little or no response. From approximately 25 to 28 days of age, there is a mild but increasingly severe reaction. The typical "young adult" response begins between 30 and 35 days of age.

Exposed nursing mothers examined during this same period exhibited a severe response somewhat greater than that observed for non-nursing females of comparable ages.

When postnatal animals were weaned at age 15 days and fed a maintenance diet for 48 hours, the response was surprising. These 23-day-old animals responded comparably to 28 to 29-day-old animals weaned at 20 days of age and treated likewise. When weaning was delayed until the twenty-fifth and thirtieth days and the animals were exposed immediately to 0.9 ppm O_3 for 48 hours, the reaction was very mild in both cases. Thus, the response of postnatal animals to O_3 appears to be related to diet and to weaning, but the basis of the differences observed is not yet understood.

Postnatal animals of the same age exposed to NO_2 respond differently from those exposed to O_3. Animals between 1 and 10 days old appear not to respond

to either NO_2 or O_3. However, between 10 and 20 days of age, the animals display an enzymatic as well as a morphological response to NO_2 but not to O_3. Edema and mild proliferation are observed, and they increase in animals exposed immediately after weaning at age 20 days. This response occurred also in animals weaned at 20 days of age and exposed for 25 or 30 days. Edema was less prominent, however, in animals older than 30 days when exposed. The observed differences in responses of animals at different ages may have important implications for human populations.

Other effects. Dr. Freeman et al. have shown that newborn rats exposed to NO_2 or O_3 develop anemia. However, exposure after age one month results in the opposite reaction, i.e., polycythemia. In parallel, the red cells change in size from overly large in the newborn to smaller than normal in the weanling. Thus, while red cells are susceptible to the same kinds of actions as lung cells, compensatory mechanisms are triggered which tend to stabilize the numbers and sizes of the cells in response to stresses on the pulmonary system.

Compensatory changes have also been observed in lung lining cells (Dr. Dungworth et al.). Upon short term exposure to O_3 or NO_2, which induces sloughing of Type 1 cells, new cells develop which are resistant to previously injurious concentrations of the gases. The basis of resistance is under investigation, with the particular aim of exploring useful ways of inducing resistance or tolerance in exposed populations as therapeutic or preventive measures.

Dr. Bernard Goldstein (New York University Medical Center) has conducted additional studies on spectrofluorescent detection of dicarbonyl compounds crosslinked to protein or aminolipids as a means of following lipid peroxidation due to ozone exposure. Characteristic fluorescence was observed in lipid extracts of freshly obtained red cells from five patients receiving the oxidant hemolytic drug diaminodiphenylsulfone (dapsone). In one individual followed from the onset of therapy, this fluorescence did not appear until hemolysis became evident after about two weeks of treatment and was most apparent in older red cells. Similar fluorescence has also been observed in lipid extracts of the red cells of rabbits receiving phenylhydrazine.

These studies are the first to clearly demonstrate in vivo red cell lipid peroxidation in the absence of vitamin E deficiency. Their potential significance in relation to studies of the toxicity of ozone and other oxidizing environmental agents is the provision of a sensitive parameter consistent with the accumulation of peroxide breakdown products. Accordingly, this parameter will be evaluated in lung subcellular fractions during chronic exposure to ozone.

Prevention of damage. Improved understanding of pathogenic mechanisms encourages one to believe that damage caused by O_3 and NO_2 at low levels can be prevented or perhaps reversed in the early stages. Dr. Dungworth et al. have discovered that peroxides formed upon lipid peroxidation are detoxified by the glutathione peroxidase system. Some enzymes in this system are inducible, forming the basis of an inducible defense system. Dr. Goldstein has demonstrated that various compounds, including allylisopropylacetamide,

para-aminobenzoic acid and chloropromazine, reduce ozone toxicity. A number of investigators have found that natural lipid antioxidants, particularly vitamins A and E, induce resistance. Dr. Milos Chvapil (University of Arizona) has shown that zinc ions and certain zinc complexes markedly stabilized lysosomes and reduce lipid peroxidation, suggesting a potential palliative effect on oxidant-induced damage. These lines of investigation are still under active pursuit in an effort to make use of the knowledge that has been developed.

Sulfur Oxides

Etiology and pathology. Several projects are being supported to gain further information about the effects of sulfur oxides on selected populations and the distribution, fate and reactions of sulfur oxides in the body. A study was initiated this year on the deposition and effects of sized sulfuric acid droplets in the lungs (Dr. Mary Amdur, Harvard University). Combinations of such aerosols with oil mists are also being tested. Results from the study are not yet available.

Last year epidemiological studies were initiated also by the Harvard scientists (Dr. Benjamin Ferris et al.). Data are being collected from selected sites to determine the effects of particulates and sulfur oxides in combination on adults and children in Watertown, Massachusetts; the Kingston-Harriman area of Tennessee; St. Louis, Missouri; and Steubenville, Ohio. While the data from these studies will not be available for some time, it is expected that definitive results will be forthcoming.

Dr. Yves Alarie (University of Pittsburgh) has continued studies on respiratory tract irritants and desensitization phenomena. Dose-response curves for exposure to SO_2 were obtained for male Swiss mice and for rats, guinea pigs, and rabbits. These three species did not show any greater sensitivity than male Swiss mice and, therefore, offer no advantage as animal models. Thus different strains of mice were tested and excellent results were obtained. A/HEJ female mice proved to be the most sensitive while DBA_2/J were the least sensitive. From these strains F_1 hybrids were obtained and tested and work with F_2 and F_1 back-crosses is now in progress.

Not only could some strains more sensitive than others be identified but the time-response pattern also varied with different strains. Repeated exposure also showed a marked difference in response, with A/HEJ showing the same degree of response while with Swiss and DBA_2/J, a gradual fading occurred until absolutely no response was seen to concentrations as high as 200 ppm of sulfur dioxide. Because of the advantages of working with one species (mice) over different species as had been proposed earlier, the development of the animal model using various strains of mice will be pursued.

More basic studies on the body distribution, fate and toxicity of inhaled sulfur dioxide have been continued. It was shown some five years ago that a fraction of the exogenous SO_2 and sulfite entering the mammalian body reacts with disulfide bonds in proteins and amino acids of plasma, forming thiolsulfonates. This has raised questions regarding the potential systemic toxicity of inhaled sulfur oxides. Dr. Albert Gunnison (New York University Medical

Center) has found that the clearance of sulfite from the bloodstream in rabbits follows a single exponential, with a mean sulfite resident halflife of about 3.6 minutes. About one tenth of that in the bloodstream was found to be nondialyzable and is believed to be bound to proteins or other macromolecules, and some interference in the activity of insulin and sulfhydryl enzymes was detected.

A major concern about the presence of sulfite in the bloodstream is its possible effect on DNA, since bisulfite has been shown to be mutagenic. This problem is being investigated by Dr. Robert Shapiro (New York University). He has found that bisulfite enters into three reactions with components of nucleic acids, e.g., deamination of cytosine, transamination of cytosine, and saturation of uracil through formation of the sulfone.

The deamination reaction is relevant to the possibility of long-term genetic damage, while the uracil saturation reaction may affect the function of RNA in protein synthesis. Research on RNA and protein synthesis shows that polyuridylic acid can be modified by bisulfite. The ability of the modified polymers to bind to ribosomes and to stimulate binding of phenylalanine tRNA to ribosomes was decreased by up to 90 percent. A natural messenger RNA was also found to be modified by sodium bisulfite. Saturation of 6 percent of the uracil residues of the RNA resulted in abolition of 64 percent of the incorporation of lysine into protein. E. Coli 70S ribosomes also exhibited a significant decrease in ability to bind to tRNA's and to incorporate amino acids into protein.

Interactions with other agents. In studies on the effects of SO_2 on viral infection, using the chicken and the Newcastle virus as experimental models, Dr. Frederik Bang et al. (Johns Hopkins University) have developed reproducible methods for determining the effects of SO_2 exposure and viral infection on the physiology of clearance.

Exposure for intermittent times each day (about 16 hours) continued over a period of seven days at 1.4 ppm SO_2 had a clear effect of slowing turbinate function by the fifth day of exposure, but sinus clearance was normal. Increasing concentrations (8, 12, and 66 ppm) had more rapid effects, and also slowed sinus clearance. Newcastle virus alone changes the clearance rate most markedly by about five to six days of infection, followed by improved clearance by day nine. Combination of NDV and SO_2 has not as yet produced a clearly synergistic effect.

Scientists at New York University Medical Center have found some indications that SO_2 in combination with carcinogens such as benzo(a)pyrene and with certain types of particulates increases the expectation of cancer. The effect is not very large and more work is required for quantitative verification.

Carbon Monoxide

Etiology and pathogenesis. A substantial number of epidemiological and basic studies are supported to provide a better understanding of the extent and nature of the hazard presented by inhalation of carbon monoxide, alone and in combination with other toxic agents.

Dr. Richard Riley and his colleagues at Johns Hopkins University have carried out extensive studies on many aspects of carbon monoxide distribution and intoxication. Epidemiological studies of cigarette smokers show that 35 to 40 percent of the hemoglobin may be in the form of carboxyhemoglobin during periods of heavy smoking. Fortunately, clearance of CO is relatively rapid so that the time during which so much of the hemoglobin is unavailable for carrying oxygen is generally short. Nevertheless, in heavy smokers, it was found that 3 to 15 percent of the hemoglobin is continually in the nonfunctional carboxyhemoglobin form.

The consequence of reduced oxygen carrying capacity is variable from inidivual to individual and is dependent upon other disorders. Patients with circulatory insufficiencies and with various forms of anemia are most susceptible to physiological disorders resulting from oxygen insufficiency, and data are now available which show significantly higher mortality rates in smokers with circulatory and blood disorders.

CO effects other than a simple reduction of oxygen-carrying capacity of the blood are also becoming evident. It has been shown that CO combines with many cellular constituents containing the heme moiety, such as muscle myoglobin, cytochrome oxidase, cytochromes A_3 and P-450, catalase and peroxidases. Dr. John T. Fales of Johns Hopkins University has examined the possible effects on muscle myoglobin further, since this compound is critical in arterial and heart function. He showed that CO decreases the isomeric tension (muscle function) by about 30 percent. Related studies carried out by Dr. Walter Ehrlich and his colleagues in dogs show that CO decreases the critical closing pressure of the heart in animals at rest and increases total arterial resistance to blood flow. This results in an increase in blood pressure and, of course, in work load of the heart.

Further insight into basic aspects of CO action on endotheloid and muscle cells was provided by Dr. Duane Wenzel of the University of Kansas. Using cultured heart cells, he found that CO-treated cells accumulate two to three times the "normal" amount of globular lipids. The effect was pH-dependent. These observations may have some bearing on the accelerated rate of atherosclerosis and other cardiovascular problems, particularly in cigarette smokers.

Dr. Zoltan Annau of Johns Hopkins has been concerned with possible behavioral effects of CO at low levels. His studies thus far indicate no detectable acute effects on rats at air concentrations of CO below 500 ppm. However, long-term studies suggest that moderate CO levels eventually lead to effects on the central nervous system. These are not due simply to the decreased oxygen-carrying capacity of the blood but may involve irreversible changes in cells and tissues.

CO transport. A saturable transport carrier for the facilitated diffusion of O_2 and CO has been identified by researchers at Johns Hopkins University in both the placenta and lung. It has been hypothesized that this carrier is cytochrome P-450, a heme-containing pigment that has been widely studied for its role in drug, hormone, and xenobiotic metabolism. Thus, the administration of diphenhydramine (Benadryl) was shown to decrease O_2 flux across the placenta in sheep, presumably by combining with the P-450. Likewise, in man a

therapeutic dose of the same drug was shown to decrease the diffusing capacity of CO by 10 percent.

In sheep, the halogenated anesthetic, methoxyflurane, was shown to be demethylated by lung microsomes and also to be able to completely block the carrier-mediated diffusion of CO across the lung in intact sheep. These results suggest that a wide variety of therapeutic and/or environmental chemicals may interfere with the transfer of O_2 across the placenta and the air-blood barrier of the lung by virtue of combining with transport molecules.

Effects of CO on drug metabolism. Dr. Margaret Hitchcock (John B. Pierce Foundation) is studing the interaction of air contaminants on selected liver functions in different animal species. She found that under conditions of adaptation to CO as determined by increases in hematocrit and hemoglobin, there also occurs intracellular adaptation to CO as shown by increases in liver cyt. P-450 content, which is also accompanied by an increase in liver microsomal protein. In vitro determinations of liver microsomal cyt. P-450 dependent oxidations showed no differences in the overall rates of metabolism when expressed in terms of wet weight but showed an expected small decrease when expressed in terms of cyt. P-450 content.

In studies on the effects of CO on in vivo drug metabolism, she found that exposure of immature male rats to CO influences in vivo drug metabolism in a variety of ways. In normal rats, low levels of CO produce an acceleration of in vivo drug metabolism, possibly by producing an increase in liver perfusion rates. The effect is less pronounced as the CO concentration increases, suggesting inhibition of cyt. P-450. This is supported by the observations in polycyclic hydrocarbon-induced rats which have an elevated cyt. P-450 content. In these rats, CO produced a dose related prolongation of zoxazolamine paralysis time at 300 and 450 ppm. Since polycyclic hydrocarbons do not alter liver perfusion rates per se, the observation that CO produces a more pronounced inhibitory effect at elevated cyt. P-450 levels suggests that exposure to CO may produce an adverse effect on the metabolism of drugs via inhibition of cyt. P-450.

Automobile Exhaust

Aerometric studies. Aerometric studies have been conducted on automobile exhaust over the past several years by scientists at Harvard University. Fixed location sampling devices for traffic officers at intersections have been replaced with personal monitors of carbon monoxide, nitrogen dioxide, respirable mass total particulates, and respirable mass lead particulates. Preliminary results from a comparison of personal and fixed sampling show that the personal sampler concentrations of respirable mass particulates were 50 to 100 percent higher than the fixed samplers at the intersections. The arithmetic mean was 121 $\mu g/m^3$, with the most frequent values from 100-125 $\mu g/m^3$.

A second year of environmental sampling of tunnel-turnpike workers has now been completed. The environmental measurements focused mainly on the toll booth operators' exposure to automobile exhaust at three toll plazas. Air sampling was conducted for nitrogen dioxide, carbon monoxide, total hydrocarbons as methane, respirable and total suspended particulates, and lead in both the

total and respirable fractions. Sulfur dioxide was also sampled since it is a prevasive pollutant. The expected seasonal variations for sulfur dioxide were found, and the concentrations were comparable with those seen in the downtown Boston area. The total suspended particulate concentrations were approximately 50 percent higher in the winter months than in the summer months and ranged from 40 to 70 percent of the total fractions. The average lead concentration in the respirable mass fraction was 13 μg Pb/m^3 with a range of 1.0 to 36.3 μg Pb/m^3.

As yet, no significant differences in mortality, pulmonary disorders or cardiovascular disease of these workers has been evident when compared to controls exposed to lower levels of automobile exhausts.

Benzene and Other Solvents

Etiology and pathogenesis. It is estimated that more than two million people are occupationally or adventitiously exposed frequently to benzene and related solvents to a substantial degree. NIEHS supports several projects in which attempts are being made to understand the toxic action of industrial solvents. Dr. Robert Snyder (Thomas Jefferson University) has developed a method for detecting very early signs of benzene toxicity. He finds that a single dose of benzene in the mouse depresses incorporation of ^{59}Fe into an early precursor of the erythrocyte in the bone marrow, with a lag time of some 12 hours. Through appropriate correlates in erythrocyte formation, precise identification of the cell type involved and the elucidation of the biochemistry of suppression is being elucidated. The depressant effect disappears after about 72 hours, which indicates that the bone marrow is not permanently damaged.

Metabolism. Metabolic studies in mice suggest that only one major metabolite is formed from benzene in the body, e.g., phenol, which is rapidly excreted in the urine as the sulfate or glucuronide conjugate. Metabolism is stimulated by pretreatment with the usual microsomal enzyme inducers or by benzene per se, which apparently acts as a self-inducer. Efforts are now being made to find antagonists and enzyme inducers that will afford protection of erythrocyte biogenesis against benzene and similar toxicants.

Suspended Particulates (Aerosols)

Model Studies

Because of the ubiquity of hazardous aerosols and their importance in man's health, a great deal of effort is being supported to elucidate the interrelationships of particle size, composition, and deposition to various respiratory disorders. Dr. John Hutchens (University of Chicago) has attempted to establish the locus and extent of retention of aerosol particles in respiratory tracts of laboratory animals. Sub-gross anatomies of the respiratory tracts are being investigated with the aid of casts, the ultimate aim being to develop the ability to interpret aerosol exposures in laboratory animals in terms of effects to be expected in man. In all models of species made so far, the sums of measured volumes of individual airway elements always fall short of actual lung volumes. The problem appears to be in making casts truly

representative of alveolar size and shape. Unless this problem can be overcome, data from models will have to be interpreted with care to compensate for these differences.

Similar modeling and deposition studies were conducted by Dr. Morton Lippmann (New York University Medical Center). In addition, a computer program has been developed to process large numbers of cast flow and particle deposition measurements. The deposition data have been used to test the validity of widely used predictive models for deposition by sedimentation and impaction, i.e., the models of Findeisen and Landahl. For spherical particles with 2 to 8 μm aerodynamic diameters, predictions from the Findeisen and Landahl relations were in good agreement with each other. However, with particles of 30 to 60 μm, the predicted values were generally much lower than the measured values, with discrepancies of up to a factor of 10.

Dr. Joseph Brain (Harvard University) has devised equipment for determining how gravity and differences in regional lung dimensions and ventilation influence particle deposition. A concentric aerosol spectrometer has been constructed which sizes aerosols according to aerodynamic diameter. Using particles labeled with ^{99}Tc, Dr. Brain was able to demonstrate differences in distribution patterns due to differences in methods of particulate administration. Groups of animals receiving intratracheal instillations demonstrated nonuniform distribution patterns with perferential deposition in the dependent portions of the lung. Groups allowed to inhale the aerosols showed more even distribution, with preferential accumulation in the apical lobes.

Lung Clearance

General. Studies were continued by Dr. Proctor et al. (Johns Hopkins University) on the respiratory tract and its clearance mechanisms. A novel technique for the collection of tracheobronchial secretions (TBS) from intact dogs has been developed. The TBS has been chemically characterized as to mucoprotein and protein composition. The presence of several plasma proteins, particularly albumin and IgG, but not all plasma proteins, suggests a selective access of the mucosecretions to the vascular compartment.

The secretory rate, composition, and rate of mucociliary transport of both an insoluble particle and a water soluble dye were all determined simultaneously following a single dose of the cholinergic drug, methacholine. At the time that the secretory rate and mucoprotein content were maximum there was complete stasis in the movement of the particles while the water-soluble dye continued to stream up the trachea unimpeded in its rate. These results have been interpreted as indicating a double-layered mucus blanket, the lower layer consisting of a protein-containing aqueous substrata upon which a gel-like layer of mucoproteins floats. Water-soluble materials are dissolved in and transported in the aqueous layer while particles remain floating on the upper gel layer. During normal secretion both the upper and lower layers are within range of the effective beat of the cilia and thus materials in either layer are effectively cleared. Following methacholine there is an outpouring of fluid, the total blanket deepens, and the surface layer is elevated away from the effective beat of the cilia. Thus, surface materials undergo stasis while the lower layer continues to be cleared. These studies have important

implications for the rates of tracheobronchial clearance of diverse particulate, aqueous, and vaporous components of the inhaled air, especially when irritants that increase fluid excretion are inhaled concomitantly.

Particle clearance. Studies on aerosol deposition in the human respiratory tract were carried out. For submicron particles of 0.02 micron diameter, which are transported only by Brownian movement, the overall deposition was found to range from 0.3 to 0.4. These results will be used to access the validity of present models derived for human respiratory deposition. It was also found that in man during oral breathing, 50 percent was removed by the nose in nasal breathing. Acute changes in small airway resistance were observed to correlate well with sub-laryngeal deposition.

A new highly simplified method for measurement of nasal clearance in humans has been developed that will be of use in field studies and children, especially in on-site studies of occupational exposure. This technique has been used to show that there is no statistically significant correlation between rates of nasal and tracheobronchial clearance in normal humans or in nasal clearance rates between monozygotic twins.

Dr. Joseph Brain (Harvard University) has done extensive studies on particle clearance and mobility in the lungs. He has shown that while much of the particle deposits in the lungs is cleared within 24 hours of exposure, considerable material remains uncleared for prolonged periods and some can be detected in the lungs even a year later. It has also been shown for the case of iron oxide that residues of the inhaled particles subsequently become stored as hemosiderin-like deposits in macrophages widely distributed throughout reticuloendothelial tissues of the body.

In the lungs, particle clearance was found to occur predominately by way of the bronchial passages. Initially the lymphatic drainage routes were seen to be little used, but in time the relative importance of this pathway increases. Particles traveling this way are often intercepted by macrophages of the pulmonary connective tissues or those in the sinuses of lymphoid tissue located there. Because these cells also maintain an expanded lysosomal compartment, it is postulated that their activity in synthesizing and turning over catabolic enzymes remains elevated. There is some evidence for this from the demonstration of considerable acid phosphatase activity long after particle exposures have ceased. Such heightened activity is considered to be an important factor in the pathogenesis of several chronic bronchial diseases.

Dr. Joseph Watson (University of Pittsburgh) has developed a somewhat novel approach in attempts to standardize mammalian test systems for the study of respiratory tract clearance. All material (particles, cells, mucus, etc.) cleared from the pulmonary system of the rat is collected by intubating the esophagus through the stomach. No surgery on any portion of the pulmonary system is involved. The method permits the direct measurement of particle clearance rate and number of macrophages that leave the lungs via the oropharynx. It was found that the hourly output of pulmonary cells in rats (after a 24-hour post-operative period) remains essentially constant over time. The linear nature of the output suggests that the method will provide a sensitive indicator of mucociliary escalator inhibition. The procedure is new

and it has not yet been extensively used in aerosol exposure studies. However preliminary data indicate good promise for these kinds of studies.

Effects of inhaled particles on macrophages. Dr. Brain has continued intensive studies on the effects of particulates on macrophages. The research continues to be guided by the premise that most respiratory disease is either initiated by, or at least complicated by, the inhalation of particles and gases. Emphysema, bronchitis, pneumoconiosis, neoplasms, and infectious disease all may be consequent to inhalation of obnoxious particles, and the severity of resultant disease may be influenced by the number of particles deposited as well as their site of deposition and their ultimate fate. The pulmonary macrophage has been emphasized since it is a central figure not only in clearance processes but in pathogenesis in some lung diseases as well.

Dr. Brain has developed a model which permits estimation of rates of particle phagocytosis in intact animals. Syrian golden hamsters received intratracheal injections of a colloidal solution of ^{198}Au (30 μm) of aggregated gold colloid. At 0, 2, 4, 6, 8, 10, and 24 hours post-exposure, the hamsters were sacrificed and the trachea was cannulated. The lungs were washed twelve times with saline and the number of cells and gold particles in each was determined. Initially, the gold washed out exponentially while the cells washed out with a different but characteristic pattern. With continued washing, the gold washout curve gradually approximated the macrophage washout curve. When the log of $(1-\lambda)$ is plotted against time, the result is a straight line, suggesting that the uptake rate is directly proportional to the amount of colloid available. Similar results were obtained from the hamsters exposed to the gold aerosol. Concurrent exposure to ferric oxide aerosol, colloidal carbon, or 72 hours' exposure to 100 percent oxygen all decreased the values of λ, demonstrating that the uptake of the test particle had been diminished by each of the types of particles and by O_2.

Dr. Sorell Schwartz (Georgetown University) is making use of in vitro cultures of alveolar macrophages to examine the processes by which macrophages incorporate particles and to determine the means by which particles block the activities of the cells and the consequences of this in immunologically specific and nonspecific areas.

Because of its known interaction with biological membranes, ostensibly by hydrogen bonding, a study was begun with silica. Four sizes of silica were used, 0.007 μm, 0.12 μm, 0.05 μm, and 36-50 μm. The first three sizes were used because of their potential ability to enter the cell. The large size was a "chemical control." Scanning electron microscopy revealed an incredible affinity of the three smaller sizes for the macrophage membrane. At a concentration of 100 mg/ml the coating of cells was complete, which apparently renders the membrane hydrophobic. After one hour of coating with silica, evidence was obtained that membrane integrity was altered. There was a loss of lactic dehydrogenase from the cell but lysosomal acid phosphatase and pinosomal sucrose ^{14}C were not lost. This appears to indicate that there are functional derangements in the cell caused by silica which precede those of lysosomal membrane toxicity.

Quartz Dust and Talc

Pathogenesis and Epidemiological Studies. A prospective study of 633 granite workers exposed to quartz dust is being conducted by scientists at Harvard University. On the basis of extensive air sampling conducted under the supervision of professor W. A. Burgess, the workers were divided into low, medium, and high exposure. It was found, as might be expected, that those workers who have the greatest exposure have the greatest loss of pulmonary function.

Pulmonary function ($FEV_{1.0}$'FVC) in talc miners and millers has been measured also in active workers for the years 1970-1973. After three years, a significant decrease in FVC can be identified. This loss appears related to occupations in the mines, but job definitions are currently inadequate to verify this. A proposal has been made to identify total job histories and assign measured dust counts per job so that high risk occupations can be identified and, if possible, a dose-response relationship generated. This is non-fibrous talc and suggests that talc should not be classified solely as a nuisance dust.

Fibrous tremolite is currently considered a hazardous contaminant of talc and its presence must be determined by laborious optical phase contrast microscopy. Although this is a very sensitive method, it may be biased low because of the very low concentration of fibers in gross talc. A method of determining tremolite in talc by infrared spectroscopy was investigated and found to provide the required sensitivity for respirable mass sampling. The procedure will be used in a planned study at the Vermont talc industry.

Asbestos

Pathogenesis and epidemiological studies. Perhaps the most important particulate from the standpoint of health hazard is asbestos. Intensive and wide-ranging investigations into this problem continue by a number of investigators. A major portion of the work is devoted to obtaining information concerning aspects of environmental asbestos disease, including disease associated with household exposure, disease among residents living near asbestos plants, and disease in the general population.

An important aspect of the studies on asbestos, and other particulates as well, is the relation of cigarette smoking to disease risk. A significantly increased risk of death of bronchogenic carcinoma among asbestos workers has been well established. Prospective observations among 370 New York metropolitan area asbestos insulation workers begun on January 1, 1963, by Dr. Irving Selikoff et al. (Mount Sinai School of Medicine) show that heavy smokers have 92 times the chance of dying of lung cancer as compared to men of the same age who neither smoke nor work with asbestos.

The cohort continues under close observation, prospectively, and the experiences of both smokers and non-smokers are being recorded to determine if a cigarette smoking-neoplasm association exists for malignancies at other sites (pleural mesothelioma, peritoneal mesothelioma, cancer of the esophagus, colon, rectum, stomach, bladder, pancreas). Initial observations suggest that

cancer of the esophagus will also be found to have such an association. Since neoplasms other than the lung are considerably less common, additional observations are required for appropriate evaluation.

An important area for environmental research has been the investigation of health effects of community air pollution. Because of the pervasive distribution of the agents concerned, it has been difficult to obtain satisfactory evidence on the question. Studies have been initiated to determine whether community air pollution might have a multiple-factor influence in the etiology of lung cancer. Should this be found to be so, it would be consistent with the hypothesis that lung cancer may result from the interaction, additive or multiplicative, of a number of agents, including cigarette smoking and community air pollution.

New studies were initiated by Dr. Selikoff and his colleagues during the past year on the biological significance of nondescript ultramicroscopic inorganic particles. Such particles are extremely numerous in tissues. They are often intracellular and sometimes intranuclear. The studies indicate their presence as atmospheric contaminants from all major pollution sources. Investigations of the microparticles in the bowel wall are also underway because of the demonstrated association between occupational exposure to asbestos and colon cancer.

Characterization of Cigarette Ash

Dr. Arthur Langer has utilized a number of physical techniques to determine the inorganic particles in cigarettes and cigarette smoke. This study is being conducted as an adjunct to the broader studies of the Mount Sinai School of Medicine group on the potentiation of lung damage by asbestos and other particulates in heavy smokers. The first year's work has produced a number of surprises. First, it appears that the inorganic residues of cigarettes produce a uniform ash regardless of the tar and nicotine content of the materials. In addition, the number of crystalline compounds in cigarette ash was unexpected. Cigarette ash consists of a number of crystalline phases which appear to range from single crystals to polycrystalline aggregates. Electron microscopic examination of the cigarette ashes indicates that a range of particle sizes exist. Microchemical analysis of some of the crystalline grains indicated that very few were "silicate" in nature. Although adventitious grains have been observed in cigarette ash by x-ray diffraction techniques, they tend to go unresolved, on a particle-by-particle basis, when examining bulk materials by electron microscopy.

Preliminary investigations into the nature of the particles in cigarette ash suggest individual grains in smoke may be characterized. This would include morphological, structural, and chemical characterization. Preliminary work concerning particle size distribution of smokers by fluctuation spectroscopy suggests that methods may be available for determining the portion of cigarette smoke retained in the human lungs after smoke inhalation.

Soot, Carbon Black, and Benzo(a)pyrene

Etiology and epidemiology. Several projects are being supported on the relationships between soot and associated benzo(a)pyrene (BP) and other carcinogens. These studies assume increased importance with increasing use of low grade fossil fuels, especially in conjunction with cigarette smoking. Both cigarette smoke and polluted urban air contain very small amounts of BP. It has been difficult to obtain direct evidence on health effects since the concentration of BP in the air of large cities is reported to vary from about 0.03 to 6.0 μg/1,000m^3 in summer and from 6 to 74 μg in winter. During the course of a year, non-smokers living in some of these cities probably inhale more BP than do heavy cigarette smokers living in rural areas, but the amount is extremely small compared to that required to produce skin cancer in susceptible strains of mice. Because of this, interest in the possible effects of small amounts of BP in cigarette smoke and urban air, the mortality from lung cancer and other causes among roofers, who have very heavy occupational exposure to BP in the air, is being investigated. Data obtained at these higher level exposures may subsequently be used to shed light on the situation regarding the general population by extrapolation.

The entire membership of the roofers union in the United States and Canada has been followed since January 1, 1960. It has been possible to trace 97.5 percent of these men through December 31, 1971. It was found that 1,798 of the men died. The cause of death was ascertained from death certificates which were obtained for all except 78 of the men who died.

Some calculations have already been completed which indicate that heavy occupational exposure to BP is associated with increased mortality from lung cancer. Of course, since the fumes from hot pitch contain various other agents in addition to BP, the findings in this study could be due to one or more of the other factors or to their combined effect.

An extensive study of the health status of printing trades workers, exposed to carbon black (as well as other substances) has been initiated involving the membership of Local #2 of the New York Web Pressmen. The mortality experience of all journeymen members of this local (approximately 1,800 men) from January 1, 1950 to December 31, 1974, will be documented. Age, year, and sex specific death rates will be calculated and compared with deaths that have actually been observed. It is expected that these studies will provide information useful in the assessment of carbon particles in other trades and occupations and in the general population.

Mechanism of benzo(a)pyrene carcinogenesis. With hamster intratracheal intubations, high yields of carcinomas were obtained with methylcholanthrene and dose-response relationships were developed by scientists at the New York University Medical Center. Inhalation of BP in combination with sulfur dioxide has resulted in the production of squamous cell carcinomas in hamsters. This finding, of significance in current air pollution problems, has been reinforced by current studies using hamster intubations of BP followed by inhalation of sulfur dioxide. Currently, the possible role of nitrogen oxides in combination with inhalation of carcinogens is being examined.

The mechanism of action of BP has been investigated. It has been shown that approximately 20 percent of the total microsomal metabolism of BP proceeds through the formation of a 6-hydroxy derivative, which then breaks down spontaneously to a 6-oxo free radical, which can be monitored by its ESR signal. Dose-response curves of both the parent BP and 6-OH BP and transformation of Syrian Hamster Embryo cells and the kinetics of transformation suggest that the 6-oxo free radical is the form of the carcinogen that binds irreversibly to the DNA and may be the ultimate carcinogen.

Workers at Johns Hopkins University have conducted a comparative study of the transformation process induced by the BP. They have found that a BP metabolite, 6-hydroxy-BP, could be one of the proximate or ultimate carcinogens of BP. This result is significant in view of recent advances in the study of the chemistry and metabolism of 6-oxo-BP radicals. It has been established that, in rat liver homogenates, the metabolic pathway to 6-hydroxy-BP constitutes about 20 percent of the total conversion of BP.

Neoplastic cell lines selected from the morphologically transformed clones induced by BP have been established. The growth characteristic of these cell lines (such as growth in soft agar, cell saturation density, etc.), as well as the tumorigenicity of lines, were investigated concurrently with research on their karyotypes.

Dr. John Little et al. (Harvard University) have studied possible interactions (synergism) between radiation and benzo(a)pyrene (BP) in an in vitro model for malgnant transformation. Transformation has been induced by BP (1.2-6.0 µg/ml medium) and by x-rays (50-400 rads). In preliminary experiments, administration of both agents has lead to an apparent synergistic effect dependent upon sequence and interval between treatments with the agents. The most effective treatment was radiation (200 rads) followed by BP (2.5 µgm) 48 hours later.

Coal Dust

Etiology and pathogenesis. Scientists at the University of Cincinnati have conducted extensive studies on the pulmonary effects of coal dust and the role of specific components of the dust in the etiology and pathogenesis of pneumoconiosis. They have shown that there are quantitative differences in chemicals extractable from bituminous coal from mines in Pennsylvania and Utah. The most striking chemical differences are in nickel and iron contents, with the Pennsylvania coal being substantially higher in both. Correlates between the incidence of pneumoconiosis in miners in Pennsylvania and Utah show that the former is significantly higher, which is in accord with the high nickel and iron contents of the coal.

Follow-up studies show that both metals are more rapidly leached from coal dusts with serum or in vitro cell culture media at physiological pH than with water. Moreover, experiments with cultured cells show that extracts from Pennsylvania coal are substantially more toxic than those of Utah coal, which again parallels the prevalence of pneumoconiosis in the two areas. Toxicity to the cells correlates with the nickel content specifically and with the inhibition of cellular respiration and uncoupling of phosphorylation in cell cultures by equivalent amounts of added nickel salts.

Although these studies must be viewed as only suggestive, other data in the literature on nickel indicate that this metal is a potent sensitizing agent when inhaled. It appears, therefore, to warrant more intensive study as a pulmonary hazard.

Studies were carried on also on the effects of coal dust in vivo on macrophages in rats. The data show that there is a substantial increase in the number of macrophages during the first month of exposure to the dust. However, phagocytic capacity, bacterial phagocytosis in particular, is substantially reduced. After three weeks of exposure, the animals also showed extensive pulmonary disease, including pneumonia and pulmonary abscesses. In animals exposed to nicket oxide dusts, the same kinds of syndromes were apparent, and the alveolar macrophages were altered in number and appearance.

Cotton Dust

Etiology and pathogenesis. Dr. William Lynn et al. (Duke University) have made significant contributions toward understanding alveolar proteinosis and chemotactic responses of human macrophages and polymorphs to cotton. They have shown that the collagen-like peptides found in patients with alveolar proteinosis are essentially the only two peptides present in isolated rabbit lamellar bodies. In addition, these two peptides are the major ones obtained by lavage from all species studied (chicken, rats, sheep, guinea pigs, and dogs). The peptides contain gly-pro-hydropro sequences throughout the entire 330 amino acid sequences, as well as the tetra oligosaccharide asparagine-glucosamine-mannose-galactose-sialic acid. The monosaccharide linkages have not been established yet.

P. W. Jeffs and David Lynn have continued work on the characterization of chemotactic factors from cotton extracts. A fraction exhibiting a yellow fluoresence under 265 nm light, which had previously been shown to exhibit chemotactic behavior toward polymorphonuclear cells, has been studied. A new solvent extraction procedure shows that this component is present in aged bracts but absent from fresh bracts and fresh leaves. This suggests that the material is not a normal metabolite of cotton. Extensive spectral studies, in combination with selected chemical transformations, has led to the identification of the chemotactic substance as 1-hydroxy-7-methoxy-4-isopropyl-1, 6-dimethyl-2(2H)-naphthalenone.

Industrial Chemicals, Intermediates, and Byproducts

Bis(chloromethyl)ethers

Etiology and pathogenesis. Exposures to chloromethylmethyl ether and bis(chloromethyl)ether have established these commerically used reaction intermediates as carcinogens. The activity of bis(chloromethyl)ether at extremely low concentrations demonstrates a very serious industrial hazard. The presence of bis(chloromethyl)ether has been detected in rivers downstream from polymer manufacturing sites and other types of industrial plants. It is apparent that these compounds are not confined to the polymer industries. Dr. Irving Selikoff et al. (Mount Sinai School of Medicine) and others have

demonstrated the presence of the compounds at very low concentrations in textile and finishing operations, and it has been known for some time that they can form in the presence of formaldehyde, chlorine and water.

Studies on this and other direct alkylating agents have been continued by Dr. Benjamin van Duuren et al. at New York University Medical Center. The high activity of bis(chloromethyl)ethers for the lung was first demonstrated by the Laboratory of Experimental Oncology. Cases of lung cancer have now been found in industries using the compound and an industry-wide epidemiological study is being conducted to develop definitive information on the health consequences of its industrial use.

Studies on the mode of action of direct-acting alkylating carcinogens are underway using ^{14}C-labelled carcinogens including β-propiolactone and bis(chloromethyl)ether in order to determine the site of binding with tissue components. Mouse skin chromatin was isolated and characterized. The material has been shown to exhibit a decrease in T_m, a virtual abolishment of template activity for RNA synthesis, and a loss of three histones, F^1, 1^o, and F^3, when reacted in vitro with the carcinogens.

Studies on structure-activity relationships of chlorinated alkylating agents to carcinogenicity by this group have led to identification of important parameters in carcinogenic activity. Dr. van Duuren now believes that these insights have been developed to a point where it is possible to predict with some confidence the carcinogenicity of compounds within this class.

Vinyl Chloride and Related Chlorides

Epidemiology. Health effects of vinyl chloride are being investigated in several laboratories, but particularly intensively by Dr. Irving Selikoff and his colleagues. Over 1,200 polymerization workers employed by three companies in the United States (Niagara Falls, New York; Midland, Michigan; South Charleston, West Virginia) have been examined. Complete work histories, vinyl chloride exposure histories, medical information, pulmonary function findings, chest x-rays, extensive laboratory study results, smoking habits, alcohol intake histories, etc. have been obtained on the workers. An initial study on mortality experience of all employees at one of the companies revealed three deaths from angiosarcoma. In addition, there was one death from portal hypertension with ruptured esophageal varices. Deaths occurred from lymphoma and leukemia, lung cancer, and other causes but these were too few for appropriate evaluation. A prospective epidemiological investigation of the mortality experience of this defined population is now being designed.

A preliminary study was initiated by Dr. Selikoff et al. to determine whether dry cleaning workers, members of the Amalgamated Clothing Workers Union in New York City, could be traced for the purposes of a cohort mortality study. It was determined that approximately 1,250 members of this union had ten years from first employment in the cleaning and dyeing industry of New York as of 1960. A 10 percent selection of these was made and tracing was attempted. Approximately 50 percent could be identified using standard tracing techniques (union records, motor vehicle and voter registration

records, telephone, etc.). However, it appeared that nearly half the group would have to be traced through the use of more difficult and time consuming tracing techniques. Thus, they have undertaken a survey of the mortality experience of those members in the union who have retired and entered a pension plan. Four hundred and ninety-five individuals did so from the time of the plan's inception on April 1, 1946 to August, 1975. Of this group, 163 are deceased and 316 are currently receiving pensions. While difficulties are known to exist with cohorts of retirees (they represent a survivor population which may have been resistant to the effects of the materials on which one is seeking information), this group nevertheless represents one for which information can readily be obtained.

Most reports of significant liver disease among vinyl chloride exposed workers have resulted from investigations of individuals exposed at high levels to vinyl chloride. While this is important, there is equal interest in whether or not liver disease may be found among individuals exposed at very much lower levels, as in workers employed in the fabrication industries, those exposed in environmental circumstances or through use of certain consumer products. This question is being approached by investigating the prevalence of liver disease among polyvinyl chloride extrusion workers in cooperation with the Textile Workers Union of America. Medical and exposure histories and data concerning their medical status, laboratory examination results, and smoking and occupation histories have been obtained on about 300 workers. The studies are expected to improve knowledge of dose-response relations in humans. Since it is expected that fewer neoplasms will be seen, the total population at risk will require much larger numbers of workers than used in other studies.

Pathogenesis. Large-scale studies of animals exposed to vinyl chloride are underway. Mice have been exposed to vinyl chloride monomer at 2500 and 6000 ppm, with some receiving oxymetholone and beta-napthoflavone for up to five months. Various light microscopy techniques failed to reveal significant changes. Electron microscopy, however, showed early alterations of the cell membrane and an increase in the smooth endoplasmic reticulum. These studies are being continued with the aim of establishing dose-disease response relationships.

Other studies concerning hepatic angiosarcomas are being extended. In a study of human material, 10 cases of angiosarcoma following vinyl chloride exposure were investigated. An attempt is being made to identify and document those features of angiosarcoma peculiar to vinyl chloride exposure. Tentatively, the following are considered to be characteristic: (1) intralobular rather than portal origin; (2) close relationship of the angiosarcoma cells to proliferated hepatocytes, which are surrounded by wide Disse spaces containing macrophages and fibroblasts; (3) spur-like projections into bloody cysts, these projections undergoing fibrosis to produce a trabecular arrangement; (4) sparseness of solid areas of angiosarcoma; and (5) loss of phagocytosis with disappearance of PAS positive granules. It is of interest that these features are also present in a case of angiosarcoma that has been seen following prolonged exposure to inorganic arsenic.

For comparison, cases of angioscarcoma of unknown etiology are being investigated, in part from the files of the Mount Sinai Hospital. Some of these cases have features identical to those considered characteristic of vinyl chloride induced neoplasms, while others are different. Some, for example, appeared to originate in portal tracts, show no relationship to hepatocytes, and to appear as large, solid nodules.

Three cases of angiosarcoma following exposure to Thorotrast were studied for comparison with vinyl chloride related angiosarcoma. Except for the Thorotrast deposits, the same morphologic features were found. Thorotrast granules were noted in atypical cells, tentatively interpreted as potential precursors of sarcoma cells while unquestionable sarcoma cells never contained Thorotrast.

Metabolism of vinyl chloride and its analogs. Dr. Benjamin van Duuren (New York University) is studying the carcinogenic intermediates of vinyl chloride and its analogs. It was initially proposed that the carcinogenicity of trichloroethylene is directly related to its metabolic conversion, probably to the epoxide. Another aspect of this metabolic conversion has been studied, namely the binding of the metabolite(s) to microsomal protein. Liver microsomes from five to nine week old Sprague-Dawley rats, ^{14}C-trichloroethylene, and the cofactors for NADPH regeneration were incubated together. The isolated, purified protein showed covalently bound trichloroethylene moieties. The extend of protein binding was decreased by addition of 7,8-benzoflavone or SKF-525A to the rat microsomes. The former is an inhibitor of the microsomal enzyme system and the latter inhibits substrates of cytochrome P-450, which is part of the microsomal oxidase system. Consistent with these findings, rats pretreated with phenobarbital had more trichloroethylene metabolite bound to protein. Addition of 3,3,3-trichloropropane oxide to the microsome mixture further increased the amount of bound metabolite. Phenobarbital activates the oxidase enzyme system, while the 3,3,3-trichloropropane oxide probably interacts with the hydrase enzymes, thereby inhibiting their hydrolysis of the trichloroethylene oxide. Finally, addition of sulfhydryl substrates (glutathione, mercaptoethanol, etc.) to the microsomes resulted in a decrease in ^{14}C bound to protein, as these nucleophiles compete with the protein for the trichloroethylene epoxide.

These studies are concerned also with whether homologs and analogs of vinyl chloride are carcinogenic and their mechanism of interaction with cellular components. The simplest analog of vinyl chloride is 1-chloropropene. A probable liver metabolite of this compound is 1-chloro-1,2-epoxy-propane. This can rearrange to the equally reactive α-chloropropionaldehyde. Both chloropropene epoxide and chloropropionaldehyde were found to react with guanosine in DMSO at 37°. Guanine yielded the same major product, which was purified by chromatography. Some preliminary spectra and analyses suggest several possible structures which will be explored during the coming year along with the reactivity of these compounds with adenine and thymidine nucleosides and with DNA. These preliminary findings indicate that hazards are associated with vinyl halides in general. Animals and man have enzyme systems which metabolize the compounds to potent carcinogens, mutagens and teratogens.

Effects of diet on the toxicity of alkyl halides. Studies on these compounds are also being carried on by Dr. Sheldon Murphy and his colleagues at Harvard University, with special emphasis on the effects of diet on toxicity. The experiments demonstrated that male rats fasted overnight (18 hours) are more sensitive than fed rats to the hepatotoxic effects of inhaled carbon tetrachloride, 2-chlorobutadiene, 1,1-dibromoethylene and 1,1-dichloroethylene (1,1-DCE). Vinyl chloride monomer (VC) and 1,1-difluoroethylene were not acutely hepatotoxic in fed or fasted rats at any concentration tested. VC when administered simultaneously with 1,1-DCE prevented the injury associated with inhalation of the latter in fasted rats. Prevention of injury may be a result of a competitive interaction since the effect was concentration-dependent.

Additional experiments have indicated that the quality of the interaction between VC and 1,1-DCE may depend upon the temporal relationships of exposure to the two compounds. Thus when exposure to 1,1-DCE followed exposure to VC (instead of simultaneous) some enhancement of hepatotoxicity of 1,1-DCE was observed. VC exposure also reduced liver glutathione concentrations.

Other recent preliminary experiments indicate that induction of liver microsomal enzymes enhances the acute hepatotoxicity of VC. Additionally, histological evidence of liver injury was consistent with the biochemical data.

Styrene

Mutagenicity tests. Dr. W. J. Nicholson (Mount Sinai School of Medicine) has found that styrene oxide is mutagenic in animals. The compound is under further study, along with its metabolites. Results of spot tests using several strains of Salmonella typhimurium were as follows: styrene did not have mutagenic activity; however, styrene oxide, which is presumed to be the first metabolic derivative of styrene, was mutagenic. All other metabolites of styrene were mutagenically inactive. It should be noted that styrene oxide is a short-lived metabolite in animal systems.

The results of these studies, which are continuing, and which will be extended to investigations of serum and urine of styrene exposed subjects, will be evaluated in light of the findings to be obtained concerning the mortality experience of styrene workers.

Epidemiological studies. For epidemiologic studies, a factory has been identified that has produced this material since the latter part of the 1940's. The work force of about 560 in this factory will be investigated in clinical studies and in an appropriate mortality study. Since the dates of birth for each of the men are known, it should be possible to calculate expected mortality rates by cause, age, and sex. Considerable information is available regarding levels of exposure to styrene monomer.

Toluenediisocyanate (TDI)

Epidemiological studies. The studies by investigators at Harvard University on molded foam workers have been continued. The data now show that there is a significant decrease in the ventilatory capacity of all workers during the working day in the plant. Environmental measurements of TDI permitted identification of four different exposure levels, hence a dose-response relationship. Significant changes occurred in the lowest group, where the ambient air level of TDI was 0.002 ppm.

Polychlorinated Biphenyls (PCBs)

General. Polychlorinated biphenyls (PCBs) are a group of chlorinated organic compounds, of which some 200 homologs are known. About 100 have been identified. Prior to the environmental concern about the persistence and accumulation of the compounds, they were used in a wide variety of applications in commerce. However, beginning in 1971, their use has been restricted to closed system dielectric applications in capacitors and transformers, in investment casting processes as heat exchangers, and as hydraulic fluids. Nevertheless, some 40 million pounds of the compounds were produced in the United States in 1974.

Persistence. PCB's are found in all major waterways and lakes of the United States and in man and aquatic organisms. Thus, they continue to be of major public health concern. In studies at Oregon State University, Dr. Virgil Freed et al. have found that the rate of evaporation of PCB isomers increases with a decrease in the number of chlorine substituents. One exception is the 4,4'-dichlorobiphenyl which shows an unusually low evaporative rate. This isomer also shows an unusually slow rate of degradation in soil. Ten isomers have been observed over a 15-month period and those containing four or more chlorine substituents show virtually no degradation. The 4,4'-dichloro isomer shows a degradation rate which is comparable with some trichloro isomers.

Pathogenesis. An effort is being made to correlate the morphological changes produced in rat liver by Aroclor 1254 with changes in the metabolism of fatty acids and cholesterol. Aroclor produced a marked inhibition of the transformation of either acetate or mevalonate to cholesterol in vitro and a significant inhibition of the transformation of acetate, but not mevalonate, to non-saponifiable fat. It appears to affect the transformation of acetate to mevalonate but not the conversion of the two compounds to cholesterol. It also inhibited the transformation of acetate to fatty acids.

Dr. Freed et al. have cooperated with personnel of the Oregon Regional Primate Research Center in a study of the effect of PCBs in rhesus monkeys. Diets containing 10 ppm of Aroclor 1242 caused a fatal illness in these monkeys, with associated characteristic pathological changes. A trichlorobiphenyl and a pentachloro biphenyl, which are major components of the Aroclor 1242 and which are retained in the tissues of the monkeys, did not produce the characteristic symptomology when fed as the pure isomers. This study was prompted by a serious epidemic of poisoning in a rhesus monkey colony in

1967-1968. The possibility of PCB intoxication was implicated. Tissues from these original animals had been stored in formalin and recently have been assayed. PCB isomers were indeed present in the liver of these animals in concentrations comparable to those found in the experimental animals. The chromatographic pattern differed from the experimental animals, however, in that the highly chlorinated isomers found in Aroclor 1260 were detected.

Metabolism. NIEHS continues to support research in several laboratories to further knowledge of the metabolism of the PCB's, the mechanisms of hepatotoxicity and carcinogenesis, and the mechanisms of long-term reproductive effects. Dr. James Allen et al. (University of Wisconsin), in addition to providing some of the first descriptions of liver pathology, have isolated several hydroxy derivatives of 2,5,2',5'-tetrachlorobiphenyl from the urine of rats and monkeys fed the parent compound. These kinds of metabolites suggest formation of arene oxides, epoxides or other cancer-producing intermediates in the body. Possible long-term effects on the newborn, particularly with respect to development and behavioral aberrations, are also being investigated.

Dr. James Stuart (University of Connecticut) is attempting to prepare hydrolysis products of pure polychorinated biphenyls and to compare chemical and enzymatic degradation routes of the compounds. Six different PCB isomers have been prepared in gram quantities but little success has been attained in preparing hydroxylated analogs by synthetic routes. Thus, small amounts of the latter have been prepared in pure form by electrochemical oxidation of the chlorinated compounds in a flow-through cell.

Development of methodology for separation of hydroxy derivatives has progressed. Five complete chromatographic separation systems have been developed and, surprisingly, the normal phase with high efficiency silica gel gives poorer separations than does the reverse phase. In general, 3,000 theoretical plates have been achieved on one 30 cm x 4 mm i.d. analytical column packed with less than 10 micron particles and between 500-800 theoretical plates on each two feet length by 7 mm i.d. column packed with 37-75 micron preparative particles.

The purified derivatives, which can be obtained in mg quantities, are being made available to other scientists for comparison with compounds obtained from metabolic studies.

Acrylamide

Etiology and pathogenesis. Dr. Sheldon Murphy (Harvard University) has studied the effects of acrylamide on pyridine nucleotide metabolism in the rat cerebral cortex. Acrylamide monomer has produced numerous human poisonings, which are characterized by peripheral neuropathy. Its use in the polymer industry and as a soil stabilizer is increasing. Acrylamide has a rather unique toxicological property in that it seems to be a strictly cumulative neurotoxin and widely differing daily dosage rates have the same end effect when a critical total dosage has been administered. Previous studies on acrylamide have focused primarily on the possible interaction of other chemicals and nutritional deficiencies on the susceptibility of animals to poisoning.

Further studies have been carried out to test the hypothesis that the amide might reduce the supply or utilization of pyridine nucleotide cofactors for energy metabolism because nicotinamide closely resembles structurally a hypothetical dimer of acrylamide and because cats, which require a dietary source of nicotinamide (as opposed to endogenous synthesis in most lab mammals), are uniquely sensitive to acrylamide neurotoxicity. The tests showed a normal flow of substrates through metabolic pathways requiring pyridine nucleotide cofactors, suggesting that pyridine nucleotide synthesis and use was normal.

Dioxins

Mechanisms of action. In extensive basic studies on the chemistry and biologic action of 2,3,7,8-tetachlorodibenzo-p-dioxin (TCDD) and related halogenated dibenzo-p-dioxins and dibenzofurans, Dr. Alan Poland (University of Rochester Medical Center) has begun isolation and characterization of putative "induction receptors," the sites to which TCDD binds to initiate expression of aryl hydrocarbon hydroxylase, using ^{3}H-TCDD of high specific activity. He is also attempting to extend the structure-activity relationship for the induction of aryl hydrocarbon hydroxylase and ALA-synthetase activity and examine the correlations with induction, receptor binding, and toxicity.

Certain observations from previous work bear on the project as a whole. TCDD is the most potent inducer of aryl hydrocarbon hydroxylase and cytochrome P_1-450 presently known, about 30,000 times as potent as 3-methylcholanthrene and other polycyclic aromatic compounds. Among the halogenated dibenzo-p-dioxins and dibenzofuran congeners of TCDD, there is a rather well defined structure-activity relationship for their potency as inducers of aryl hydrocarbon hydroxylase. To the limited extent toxicologic data are available on these congeners, there is a precise correspondence between their potencies as inducers and their toxic potency. The phenotypic trait of aromatic hydrocarbon responsiveness, that is, the capacity of polycyclic hydrocarbons to induce aryl hydrocarbon hydroxylase and cytochrome P_1-450, is inherited as an autosomal dominant trait in crosses and backcrosses among the prototype inbred strains of mice, C57BL/6J and DBA/2J. While DBA/2J fail to respond to 3-methylcholanthrene (or other polycyclic aromatic compounds used as inducers), TCDD induces DBA/2J and all inbred strains of mice tested. The aryl hydrocarbon hydroxylase activity and cytochrome P_1-450 induced in nonresponsive mice by TCDD or in responsive strains by TCDD or 3-methylcholanthrene are indistinguishable. In nonresponsive strains of mice the log dose-response curve for the induction of hepatic hydroxylase activity by TCDD is shifted to the right: ED50 for nonresponsive strains $\geq 1 \times 10^{-8}$ mol/kg and responsive strains ED50 = 1×10^{-9} mol/kg. This evidence suggests that the mutation in nonresponsive strains resulted in an "induction receptor" with a diminished affinity for inducing drugs.

The available evidence suggests that TCDD is not metabolized or is metabolized very slowly (< 5 percent per week). Thus measurement of radioactivity in tissues of a laboratory animal after the administration of TCDD, for all practical purposes, is a measure of the parent compound.

Experiments in the past year have been directed at locating, isolating, and characterizing the putative "induction receptor" that is the site which binds TCDD, and the ligand-macromolecular complex which initiates the sequence of events which ultimately result in the expression of aryl hydrocarbon hydroxylase and cytochrome P_1-450.

Since TCDD is not appreciably metabolized and is probably taken up by passive diffusion, localization of TCDD in the liver after low dose administration of labeled TCDD should reflect receptor affinity. It was found that six hours after an administration, hepatic uptake in a C57BL/6J was 19.5 percent $\pm$ 2.2 percent of the total dose administered. In DBA/2J (nonresponsive strain) uptake was 14.2 percent $\pm$ 1.3 percent and in the hybrids, B6D2F_1/J hepatic uptake was 14.2 percent $\pm$ 2.3 percent. Further studies showed this difference in hepatic localization of TCDD between C57BL/6J and DBA/2J mice was present from three hours after administration to 14 days. 2,3-Dichlorodibenzo-p-dioxin is a congener which does not induce AHH, and presumably has no receptor affinity. Administration of this congener, ^{14}C-labeled, resulted in a very low hepatic uptake (1-2 percent) which was the same in C57BL/6J, DBA/2J, and B6D2F/J mice. These data show that uptake-related structure and recipient species are critical factors in toxicity.

Efforts have been made to determine the subcellular localization of the putative receptor. After *in vitro* incubation of ^3H-1,6-TCDD of high specific activity (52.5 Ci/mmole) with liver homogenate and subsequent cell fractionation by centrifugation, 35-40 percent of the radiolabel was found in the "nuclear" fraction, 25 percent in the "mitrochondrial" fraction, 20-30 percent in the "microsomal" fraction, and only 10-15 percent of the drug was present in the cytosol. When animals were pretreated *in vivo* with a large dose of unlabeled TCDD (3×10^{-7} mol/kg) and ^3H-TCDD was added to the liver homogenate *in vitro*, the radioactivity in the cytosol was largely displaced. *In vivo* pretreatment with unlabeled inactive congeners (dibenzo-p-dioxin, 2,7-dichlorodibenzo-p-dioxin) failed to displace the fraction of ^3H-TCDD in the cytosol. These experiments support the idea of a high affinity, low capacity binding site for TCDD in the cytosol. In liver supernatant of C57BL/6J, only one class of binding site was identified with a K_D of 2.7×10^{-10}M and n of 84 fentomoles/mg protein for ^3H-TCDD. The equilibrium dissociation constant of the ^3H-TCDD-cytosol binding macromolecule is $2\text{-}3 \times 10^{-10}$M in C57BL/6J mice and Sprague Dawley rats compared with *in vivo* ED_{50}s for induction of 10×10^{-10} mol/kg and 8.5×10^{-10} mol/kg in these species, respectively. There is no specific binding observed for tritiated dibenzo-p-dioxin, a congener which does not induce or block induction of aryl hydrocarbon hydroxylase by TCDD. In the nonresponsive strains (prototype DBA/2J) the specific binding was much less over the concentration range studied. It is difficult to saturate the receptor because of the limited solubility of TCDD. Congeners of TCDD, which are potent inducers of aryl hydrocarbon hydroxylase *in vivo* (e.g., 2,3,7,8-tetrabromodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran) compete with ^3H-TCDD for high affinity specific binding sites in the liver cytosol. Inactive congeners which fail to induce hydroxylase activity (e.g., dibenzo-p-dioxin, 2,7-dichloro-dibenzo-p-dioxin) do not compete with the specific binding of ^3H-TCDD.

A large series of dibenzo-p-dioxins, dibenzofurans and other related analogs of high purity have been synthesized. These compounds and several radiolabeled dioxins have been provided to a number of government, university, and industry groups involved in chemical and biological research of chlorinated dibenzo-p-dioxins and dibenzofurans in the environment: USDA; Bureau of Fisheries; Department of Interior; University of Missouri; Food and Drug Administration; Environmental Protection Agency; and others.

Investigations on the mechanism of induction of aryl hydrocarbon hydroxylase and the putative induction receptor for this enzyme are of considerable interest as a model of regulatory gene control. The mutation in nonresponsive mice, an altered affinity in the induction receptor, is one of the few clearly documented regulatory mutations in higher eukaryotic organisms, and further study of this locus should provide further insight into genetic regulatory mechanisms. Aryl hydrocarbon hydroxylase activity and cytochrome P_1-450 have been shown to be responsible for polycyclic hydrocarbons and acetylaminofluorene hydroxylation to their ultimate carcinogenic metabolites. Clearly, understanding of the control of this enzyme bears on models of chemical carcinogenesis.

Surfactants

Properties vs. toxicity. Dr. Joseph Arditti (University of California) has conducted a series of interesting studies on the biological effects of surfactants (detergents), using several species of plants and cultured cells as test organisms. Three series of surfactants were tested: (1) sulfobetaines; (2) sodium alkyl sulfates (anionic), and (3) ethoxylated higher alkanols (nonionic).

In general, nonionics and ionics (sulfobetaines) containing the highest alkyl group (lipophile) were least phytotoxic. The same also holds true for nonionics having the highest ratio of EO to alkyl chain. In latter homologous series, such members would also be of highest molecular weight and HLB numbers, and be the most water soluble. This, however, results in lower wetting and detergent properties.

The series of surfactants will be extended by synthesis of additional compounds in the direction suggested by the data and indicative of lower phytotoxicity. Surfactants with lower lipophiles or HLB numbers alter the solution properties more efficiently than members having higher alkyl chains (lipophiles) or HLB values. These more effective surfactants may, therefore, influence biological events at threshold concentrations, and in many cases this may be the reason why they promote growth at the lowest concentration. At higher concentrations, the more efficient surfactants could reasonably be expected to upset the micellar organization within cells as one reason for toxicity.

Phthalates

Pathogenesis. Dr. Robert Rubin et al. (Johns Hopkins University) have found that the intravenous administration of a detergent-solubilized dosage form of the plasticizer, di(2-ethylhexyl)phthalate (DEHP), results in pulmonary

damage that is characterized by an inflammatory and edematous response of the interalveolar septa of the lung. In rats, respiratory death is seen at an LD_{50} dose of 250 mg/kg. Microscopic damage in the lung can be seen at doses down to 40 mg/kg. The pulmonary pathology is quite similar to other forms of "shock" or "wet" lung and is associated with massive falls in arterial blood pressure. The pathology and lethality can be prevented by pretreatment with the anti-inflammatory steroid, methylprednisolone. These results raise concern about the migration of solubilized DEHP from vinyl plastic bags into stored blood products to be used for transfusions in humans.

Persistence and biodegradation. Dr. Douglas Ribbons (University of Miami) has continued efforts to isolate and characterize bacteria that degrade phthalates. Numerous enrichment cultures with the phthalic acids and their esters as carbon sources have been made. Little difficulty was encountered in the isolation of several pure cultures of aerobic bacteria. Of five cultures selected for more detailed study, one (EN5a) would grow on ortho-, iso- and ter- phthalate; ortho-phthalate had been used for the initial enrichment. These strains have been characterized by nutritional and biochemical tests. A variety of phthalate esters have also been successfully used to isolate aerobic bacteria. The esters employed were: dimethyl, diethyl, dibutyl, di-isobutyl, dioctyl, octyl-decyl, di-isodecyl, di-2-ethylhexyl and butyl-benzyl. These bacteria have not yet been identified.

ortho-Phthalate oxygenase activity can now be reproducibly obtained from Ps. putida. Activity is markedly affected by the growth phase. The assay developed is the measurement of NADH and phthalate dependent O_2 consumption. The apparent losses of activity during conventional protein purification have now been retrieved by the recombination of two protein fractions. Both protein fractions have been substantially purified to give red and yellow solutions but they are not yet homogeneous preparations. However, the later enzymes of the pathway of o-phthalate oxidation have been removed and the isolation of the initial product of phthalate oxidation should now be possible. The stoichiometry of the reaction is (moles): o-phthalate, 1:NADH, $1:O_2$, 1. The product, based on its chemical reactivity and absorbtion spectrum, appears to be a dihydrodiol.

Biodegradation of phthalates by microbes is being investigated by Dr. Barrie Taylor (University of Miami). Water and sediments were collected from eleven stations in the Miami River to the Gulf Stream. The major esters in the water were DEHP, dibutyl phthalate (DBP), butyl benzyl phthalate (BBP) and butyl glycocylbutyl phthalate (BGBP) at cumulative concentrations of 1-2 ppb.

Attempts were unsuccessful to develop a plate-clearing assay for phthalate-esterase activity using a medium with an ester, e.g., DMP, DEP, or DEHP, dispersed by sonication prior to solidification. As an alternative, the Most Probable Number (MPN) Method will be tried separately with two esters: DBP, relatively soluble ester with a straight-chain alcohol and DEHP, a poorly soluble ester with a branch-chain alcohol. Microbes were readily enriched aerobically from marine muds with ten different esters. Four pure cultures of bacteria were isolated which definitely grow on the enriched substrate (DMP, DEP, DEHP, BBP) and at least one representative isolated on each of the ten esters is anticipated by the end of the year. The DMP isolate

oxidized o-phthalate, but not methanol, whereas the other isolates attacked the alcohol but not the acid. The organisms showed broad, but different, specificities towards phthalate esters. These results, and isolation of aerobic bacteria on the three phthalic acids, indicate that phthalate esters are potentially biodegradable in aerobic marine sediments.

Enrichments for photosynthetic bacteria with phthalic acids and for nitrate-respiring bacteria with the m- and p-isomers were negative. Mixed cultures of bacteria, enriched from marine muds, grew anaerobically with denitrification on o-phthalate. The anaerobic catabolism of o-phthalate was directly proportional to the nitrate added (nitrite was ineffective). o-Phthalate and benzoate were anaerobically oxidized, but not the aerobic intermediates, protocatechuate and catechol. All four compounds were attacked aerobically, after a lag period, by cells grown anaerobically. Aerobic, but not anaerobic, oxidation of o-phthalate by anaerobically-grown cells was inhibited by chloramphenicol, indicating that the aerobic and anaerobic pathways utilize different enzymes.

Cells, grown either aerobically or anaerobically, accumulated α-hydroxymuconic semialdehyde (HMSA) during aerobic catabolism of o-phthalate or benzoate. A bacterium, isolated from the mixed culture, grew aerobically on o-phthalate and also accumulated HMSA. This isolate is probably responsible for the anaerobic catabolism of o-phthalate but anaerobic growth is restricted because it only reduces nitrate to nitrite, which is toxic. Another organism, isolated from the mixed culture, grows by denitrification on nutrient broth (not o-phthalate) containing nitrite. The two isolates have not yet been grown together. The main conclusion from the results with the mixed culture is that o-phthalate is subject to anaerobic microbial degradation.

Hexachlorophene

Toxicity mechanisms. Studies by Freed et al. (Oregon State University) of the mode of action of hexachlorophene have been focused on the inhibitory effect on dehydrogenase enzymes and membrane effects. Previous studies demonstrated that hexachlorophene inhibits a series of pyridinenucleotide-linked dehydrogenase enzymes. Inhibition was observed with micromolar concentrations and kinetic studies indicated cooperative inhibition. Analogs of hexachlorophene also inhibited these enzymes, with increased inhibition being observed with increasing numbers of chlorine substituents in the molecule. The extent of inhibition could not be correlated with partition coefficient or the pK of the compounds; however, the kinetics of inhibition by all analogs was similar. More extensive studies with glucose-6-phosphate dehydrogenase indicated that hexachlorophene interfers with initial binding of the substrate to the enzyme, as well as product dissociation. Spectral data indicate that hexachlorophene binds to the enzyme, the substrate, and the coenzyme. There was some evidence that hexachlorophene exhibited surfactant properties in that the torula yeast enzyme dimer was dissociated into an inactive monomer.

Among a number of membrane effects produced by hexachlorophene is a marked uncoupling of oxidative phosphorylation. In exploring the possible mechanism for this effect, it was observed that low concentrations of hexachlorophene produced a large increase in the premeability of mitochondrial membranes to hydrogen ions. The premeability of potassium ions was not affected. Complementary studies with synthetic phospholipid bilayers showed that nanomolar concentrations of hexachlorophene increased the conductance of the bilayer by a factor of 10^5. The response was linear over a wide concentration range and the maximum response was observed at pH 8. Hexachlorophene increased the permeability of the synthetic bilayer to both hydrogen and postassium ions; however, the response of the hydrogen ion was some 80,000 times greater than that of the potassium ion. It is suggested that the hexachlorophene may act as an anion charge carrier, transferring cations across membranes.

Heavy Metals

Heavy metals constitute a major class of pollutants. Though the major emphasis in the past has been on lead and mercury and their compounds, investigations are increasing on cadmium, chromium, copper, manganese, arsenic, and some of the rare earth elements.

Mercury

Epidemiological studies. Dr. Thomas Clarkson (University of Rochester) has continued his program on human occupational exposure to elemental mercury vapor. In studies of five volunteers, quantitative information on the degree of retention of inhaled mercury vapor in man has been obtained. Following almost complete retention of vapor in the lung, it is excreted from the body slowly, with a biological half time of about 58 days. This indicates that workers occupationally exposed to the vapor will continue to accumulate the metal for about a year. From the practical point of view, blood and urinary mercury levels would not be useful indices of exposure until the worker had been exployed for a year.

Studies on volunteers have revealed a new pathway of excretion of mercury in man. Significant amounts (about 7 percent of the inhaled dose) were excreted from the lungs as volatile mercury. This confirms previous work at Rochester on experimental animals and may explain the findings of Bramen in Florida of elevated levels of mercury in the air of crowded rooms. The data suggest that measurement of exhaled mercury could be used as an index of recent exposure.

Induction of metallothionein by Hg. Studies on experimental animals and on volunteers may explain the phenomenon of tolerance to mercury vapor reported by Goldwater and his colleagues in the 1950's. They noted that some workers after long-term exposure to the metal showed no signs of ill health. It has been found that repeated exposure of animals to metallic vapor leads to the induction of metallothionein. This protein may be the basis of tolerance since it forms tight complexes with Cd and inorganic Hg and protects tissues from the toxic effects. Levels of the protein in kidney tissue are dramatically increased by exposure to mercury vapor.

Dr. Clarkson and his colleagues have found evidence of the induction of the protein upon exposure to elemental mercury vapor in the kidney but not in the liver. Fractionation of a soluble protein extract from rat kidney on Sephadex G-75 columns reveals that more than 70 percent of the mercury is bound to a 10,000 mol. wt. protein. This protein showed significant absorption at 254 mu, which is a characteristic of metallothionein. No cadmium was detected in the kidney homogenate or in the subcellular or Sephadex G-75 fractions, or by the atomic absorptive method. These results suggest that mercury, deposited in kidney after repeated exposure of rats to the vapor, binds to a protein having characteristics similar to those of metallothionein.

A partial purification was attempted of the 10,000 molecular weight proteins from rat kidney after repeated exposure to mercury vapor following the methods of Cherian. The 10,000 molecular weight fraction from Sephadex G-75 filtration was subjected to isoelectric focusing using ampholines, pH 3-6. Two protein peaks and corresponding mercury peaks were observed with isoelectric points at pH 4.5 and 4.9. These results are similar to those reported previously for isoelectric focusing of metallothionein induced in rats by pretreatment with cadmium salts.

These experiments were made at high vapor concentrations (about 4 mg Hg/m^3). Occupational exposures are at least an order of magnitude lower than this. However, exposure times in the experiments were brief (2 hours), and the animals were exposed for not more than eight days. The induction of metallothionein-like protein in kidney tissue and the binding of mercury to these proteins may be one explanation for the persistence of mercury in urine long after cessation of occupational exposure and for the reports of human tolerance to mercury vapor in long term occupational exposure.

Neurological effects of Hg. Dr. Werner Niklowitz has continued studies designed to gain an understanding of the effects of mercury on the central and peripheral nervous system. Experimental mercurialism was produced in different species of mammals (guinea pig, rabbit, rat, mouse, cat, and nonhuman primates). Tissue changes have been examined with the light and/or electron microscope. The nervous system showed pathological changes in the peripheral nerves and posterior spinal roots, Schwann cells and myelinated axons, or lesions in the central nervous system with degenerative processes and loss of nerve cells in the cerebellum, calcarine fissure, brain stem, and cortex.

Extensive studies of a similar nature have been carried out by Dr. Karle Mottet (University of Washington). He has attempted to define further the effects of congenital chronic low-dose exposure of the rat fetus. A single dose of 2 mg methylmercury hydroxide/100 gram b.w. was injected subcutaneously on the fifth day of pregnancy and the rats were serially sacrificed one to 10 days later. The mercury burden of the various regions of the intestine was compared with the liver and blood levels. Blood, liver and intestinal Hg reached maximal levels within 24 hours then gradually declined. More significant was the fact that the duodenal level was consistently higher than that of other regions of the intestine and liver and about 1/3 the level in blood. These observations do not lend credence to the observation by other

workers that Paneth cells are a principal route of excretion of inorganic mercury. Paneth cells are more numerous in the ileum than duodenum and are normally absent from the colon. Detailed histologic and ultrastructural study of the rat Paneth cells reveals degeneration and necrosis rather than evicence of Hg excretion. Radioautography did not indicate concentration of mercury by the Paneth cells.

Another approach to the probem was to study the effects of congenital and neonatal (maternal milk) methylmercury in the differentiation of Paneth cells in newborn rats. Fourteen pregnant females were given 2 mg CH_3HgOH s.c. in divided doses throughout pregnancy and during the first month after delivery. Electron microscopy revealed that Paneth cell differentiation was unchanged, and there was no decrease in fetal Hg burden following Paneth cell differentiation. Chronic low dose Hg did produce some degeneration and necrosis as observed above.

During the past year, testing was completed of a group of adolescent Rhesus monkeys given small daily doses of methylmercury to establish a chronic dose-response baseline. The studies indicate that chronic exposure for up to 1 1/2 years at blood levels below 2.0 ppm do not produce obvious symptoms in adolescent Rhesus monkeys. Visual fields, eye-hand coordination, gross motor coordination, and diagnostic blood tests were all normal. When the blood levels was gradually raised between 2.0 and 2.5 ppm, all of the animals exhibited a rapid onset of neurological symptoms. Although the exact constellation of disorders differed somewhat between animals, there were radical changes in emotional behavior, anorexia, loss of fine control of the digits, and inefficient mastication. In chronically exposed animals, gross motor incoordination occurred, but was relatively subtle, whereas animals acutely poisoned with large doses of methylmercury exhibited profound incoordination of locomotion with relatively unaffected fine control. These differences in the loss of motor control are correlated with the distribution of neuropathological lesions. Until the last days that the behavior of the monkeys could be tested, there was no evidence of loss of peripheral visual fields. Continuing behavioral tests of monkeys chronically exposed to methylmercury will concentrate on assessment of subtle changes in emotional behavior, monkey "personality" and the acquisition of complex learning tasks (intelligence).

Dr. Richard Manalis (University of Cincinnati) has shown that 1.0 μM mercury increases the amount of transmitter released from the nerve terminal following the arrival of an action potential and that the large increase in the EPP amplitude is due principally to this potentiating effect of the low concentration of mercury on evoked transmitter release. At low concentrations of $HgCl_2$, mercury dithiolate bridges form within the presynaptic nerve terminal as the presynaptic action potential arrives. Under these conditions, mercury ions are presumed to act like calcium ions with regard to the evoked release of transmitter. However, as the concentration of mercury ions is increased, or the exposure time to a relatively low concentration of mercury ions is increased, blockage of transmitter release occurs, presumably because the bonds between sulfur and mercury are relatively irreversible and do not allow for thiol groups to be reformed as they would in the presence of calcium ions.

Teratological effects of Hg. Dr. Virgil Ferm and his colleagues at Dartmouth Medical School have completed a study on the distribution of ^{203}Hg-labeled mercury salts (mercuric acetate, mercuric nitrate, and phenylmercuric acetate) in maternal, placental and embryonic tissue 24 and 96 hours after maternal administration of the salts on the eighth gestation day in the hamster. This work shows that the three compounds cross the placental barrier in significant amounts.

One variable which influences the teratogenicity of inorganic mercury in the hamster is the time of its administration during the critical period of organogenesis, i.e., days seven to nine of gestation. This factor was studied by injecting pregnant hamsters with 4 mg/kg mercuric acetate at one of six times (day 7, 8 p.m.; day 8, 8 a.m., 2 p.m. or 8 p.m.; day 9, 9 a.m. or 2 p.m.). The results indicate that the incidence of embryonic death and the incidence and types of external malformations vary with the time of injection. The study of internal and skeletal malformations will be continued this year.

Studies by Dr. Schroeder (Darmouth Medical School) on mice exposed to methylmercury show that brain weights are significantly reduced by the compound, as is protein. RNA was significantly lower in controls than in mice fed methylmercury and barium, calculated both on the per g tissue basis of the whole brain and per unit weight of tissue. DNA was lower in methylmercury and barium-fed mice and higher in cadmium-fed mice. Cerebrums of neonate mice showed similar changes. Practically all of the mercury (9 μg) was associated with the RNA-protein fraction, with a little, 0.5 μg, in the DNA fraction. Fractionation of liver, lung, heart, spleen, and kidneys of merthylmercury-fed mice into nuclear, mitochondrial and soluble protein fractions revealed that most of the mercury was in the nuclear fraction, except in the kidney where half was in the mitochondrial fraction.

Dr. Sheldon Sparber (University of Minnesota) has found that exposure of older embryos of chicken eggs to methylmercury results in lower hatchability than exposure of the young embryo. When functional consequences of exposure to equi-effective "killing" doses are compared, it turns out that exposure early in development renders the chick more sensitive to chemically and electrically induced seizures and less capable of learning tasks when compared to controls of older embryos injected with the same or lower doses at a later stage of development.

Interaction between Hg, glutathione, and other metabolites. Dr. Sheldon D. Murphy and his colleagues (Harvard University) have conducted studies on the effect of glutathione depletion on tissue deposition of methylmercury in rats. Diethylmaleate (DEM) and other compounds capable of being metabolized to mercapturic acid derivatives were administered to rats in order to deplete tissue stores of glutathione (GSH). Deposition of mercury was significantly decreased in kidney and erythrocytes of depleted animals when methylmercuric-(^{203}Hg)-chloride was administered during the period of maximal depletion of GSH. Increasing doses of DEM resulted in steadily decreasing GSH concentrations in brain, kidney, erythrocytes, and liver, which were accompanied by decreases in mercury deposition in kidney and brain. Increasing the time interval

between DEM and subsequent methylmercury administration resulted in correspondingly greater concentrations of mercury in kidney that were associated with recovery of renal GSH levels. Disodium maleate was also effective in lowering GSH levels and reducing mercury deposition in kidney and erythrocytes. A second dose of disodium maleate, administered 24 hours after the first, was less effective than the first in depleting renal GSH and reducing mercury deposition. However, a second dose of DEM was equally as effective as the first. A high degree of correlation was found between renal mercury accumulation and renal GSH concentration when data from all depletion experiments were considered. The results suggest that renal GSH may be a determinant in the deposition of mercury in the kidney.

It is of interest to note that glutathione has recently been shown to occupy a central position in the mechanism of amino acid transport, a process that is especially active in kidney patients with genetic deficiencies in GSH-synthesizing enzymes. Such patients have subnormal quantities of GSH in their tissues and present a generalized aminoaciduria. Furthermore, the cycle of reactions regenerating GSH used in amino acid transport (the γ-glutamyl cycle) is dependent upon metabolic energy in the form of ATP, and inhibition of renal mercury accumulation by 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, has been demonstrated.

Some preliminary experiments were conducted on the influence of amino acids and metabolic inhibitors on mercury uptake by rat kidney cortex slices in vitro. Although the results of addition of diethylmaleate on mercury uptake in vitro were equivocal, they suggested that complexing of mercury with the amino acid cysteine enhanced mercury uptake by kidney slices. Anaerobic conditions, addition of dinitrophenol, or reduction of incubation temperature from 37° to 25° reduced the uptake of mercury. These findings support the concept that mercury uptake in the kidney proceeds by two processes, one energy-dependent and linked to amino acid metabolism and one which is not energy-dependent.

Interactions may occur between mercury and other chemicals to which man is exposed, thus complicating assessment of hazard. Previous studies at Rochester have established that moderate doses of alcohol can affect the retention and excretion of mercury vapor. It has now been demonstrated that alcohol can protect animals against the toxic action of inhaled vapor. Alcohol may be one of many chemicals that act by inhibiting the conversion of elemental mercury to its toxic form, ionic mercury (Hg^{++}). The herbicide aminotriazole also acts in this way.

Hg in fish and marine mammals. High levels of mercury were found by Buhler et al. (Oregon State University) in fish taken from Antelope Reservoir in Eastern Oregon. It is assumed that the mercury is a legacy from old gold mining activities. The concentration of mercury in fish placed in the reservoir increases at a linear rate for a period of five months. The concentration in the tissues becomes constant at nine months, even though the fish continue to grow and the total body burden increases. In less than four months the concentration of mercury in the tissues exceeds 0.5 ppm. Attempts are being made to interpret these observations by determining the

contribution of food and water to the tissue concentrations of mercury in Rainbow trout. It has been observed that the mercury in the water or the food provides a constant rate of increase of tissue mercury and that both sources become additive when imposed simultaneously. Fish taken in Cottage Grove Reservoir also show high levels of mercury; however, in this case the origin of the mercury appears to be from natural sources, since there are significant deposits of mercury-containing ores in the area. Species common both to the tributaries and to the reservoir tend to show higher concentrations when taken from the reservoir. The concentration of mercury found in the tissue appears to be a function of the age of the fish. The concentration of mercury in the sediments is somewhat variable; however, the proportion of the methylmercury is relatively constant, ranging from 2 percent to 4 percent.

Marine mammals are carnivores at the top of the marine food chain and observations on sea lions taken along the Pacific coast of the United States have indicated that these species accumulate high levels of mercury as well as some chlorinated hydrocarbons. Analyses also have been carried out on samples of marine mammals taken from areas adjacent to the Arctic icepack. In contrast to marine mammals taken along the Pacific coast, the levels of mercury and chlorinated hydrocarbons are quite low. By contrast, the cadmium levels in tissues of these particular animals are relatively high.

Lead

Screening methods. Lead in its various environmental forms continues to be a major concern and numerous projects are supported by NIEHS on various aspects of the problem.

Studies by Dr. Sergio Piomelli (New York University Medical Center) are directed along three lines: to develop techniques for measurements of free erythrocyte porphyrins (FEP) on blood samples collected on filter paper, to evaluate the clinical significance of FEP elevation in regard to both Pb intoxication and Fe deficiency anemia, and to clarify the molecular mechanisms of protoporphyrin binding in both Pb intoxication and erythropoietic protoporphyria.

A simple technique has been developed to measure FEP as well as hemoglobin from blood samples collected on filter paper, with a degree of precision equal to the previously developed wet method. Using precalibrated papers, large screening programs have been initiated. Approximately 7,000 children have been screened in the pediatric clinics of Bellevue Hospital. The New York City Board of Health has adopted the filter paper technique to screen 125,000 children in the last year.

The correlation between FEP level and Fe deficiency anemia has been evaluated by comparing FEP levels with both transferrin saturation and Hgb level in children in whom Pb intoxication could be excluded with certainty (blood Pb < 30 μg/dl). The FEP levels increase exponentially with a decrease in both transferrin saturation and hemoglobin. Thus an elevation of FEP appears to be a biochemical indicator of Fe deficiency which becomes positive at a stage consecutive to the decrease in transferrin saturation. Therefore, FEP is a particularly useful indicator of Fe deficiency in children below six

years. An extreme elevation of FEP ($\geq$ 190 µg/dl) is observed in all cases of definite Pb intoxication (blood Pb $\geq$ 80 µg/dl) requiring immediate treatment. Accordingly, all children with clinically significant Pb poisoning also have an elevation of FEP $\geq$ 160 µg/dl). These findings indicate that significant metabolic effects of extensive Pb absorption may occur at blood Pb levels previously considered harmless and that a minority of children with moderate elevation of blood Pb (35-59 µg/dl) have no elevation of FEP. Reexamination of these children often fails to confirm the moderate elevation of blood Pb, suggesting either a transient elevation or technical analytical error. However, the true physiological significance of those rare cases in which the biological indicator (FEP) disagrees with the clinical indicator of absorption (blood Pb) remains to be established.

The FEP test is also particularly useful to dermatologists to detect the rare genetic disorder erythropoietic protoporphyria. A study of the metabolic difference between the elevation of protoporphyrin in Pb intoxication and erythropoietic protoporphyria has been directed toward elucidation of the lack of photosensitivity in Pb intoxication. This study has demonstrated that protoporphyrin leaks in vivo and in vitro from the erythrocytes in erythropoietic protoporphyria within a short period after entering the circulation and induces photosensitivity in the skin. In Pb intoxication, protoporphyrin remains firmly bound to the erythrocyte throughout its life span and thus cannot diffuse into the skin. Biochemical data indicate that protoporphyrin is firmly bound to the available heme sites in Pb intoxication, while in EPP it is loosely bound to the surface of the hemoglobin, at a site that bridges the α and β chain. These studies have also led to the demonstration that in Pb intoxication, protoporphyrin is chelated by Zn, which alters the fluorometric spectrum.

A rapid method of screening for lead poisoning is being investigated by Dr. Charles Colbert (Wright State University). Use is made of a quantitative computer/scanner system for rapid screening of radiographs of lead in bone. Some inconsistencies in the procedure have been eliminated by use of ash weight and density measurements in the computer program. This has led to more sensitive detection of lead in bones than previous methods.

Neuropathology. Current studies by Dr. Werner Niklowitz (Indiana University Medical Center) have shown that tetraethyl lead induces hydropic changes in nerve cells, with a specific pattern of degeneration known as neurofibrillary nerve cell degeneration. CNS neuron degeneration produced in rabbits resembles Alzheimer's neurofibrillary degeneration of CNS neurons in humans. Enzyme studies in fractions of three brain areas (frontal cortex, cerebellum, hippocampus) revealed that alkaline phosphatase was decreased in all areas studied, whereas the activity of acid phosphatase was increased in the hippocampus and frontal cortex but decreased in the cerebellum.

To substantiate recent findings concerning the interference of Pb with essential trace metals of the brain, brain tissues of control as well as TEL treated animals were analyzed every second month for Fe, Cu, Zn, and Pb. These year-long investigations established that there is a seasonal shift of essential trace metals in the untreated control brains. The interference of Pb with essential trace metal is indicated by an almost persistent decrease

of Zn and slight increase of Cu levels. Fe levels are decreased by Pb during periods with high Fe content of the brain areas and increased during periods of normally low Fe content. These data may explain why children up to three years of age are most severely affected by lead and why the high incidence of lead encephalopathy and death occurs in the hot summer months.

Dr. Richard Manalis is continuing work on the effects of heavy metal ions on the chemical synapse. In previous experiments it was shown that Pb^{++} blocks the evoked release of transmitter from presynaptic nerve terminals and that this effect is antagonized by calcium (Ca^{++}). In more recent experiments, this antagonism has been defined more precisely. Further studies on dose response show that the slope of the curve changes following the "shock" of administration of 1.0 μM lead Ringer solution. Modified Lineweaver-Burk plots of the data from these experiments suggest that the inhibition of lead is neither a competitive nor a non-competitive one but, rather, an uncompetitive inhibition. That is, lead ions apparently act at a site within the presynaptic membrane which is different from the calcium receptor site.

Studies on the role of lead in brain dysfunction are being conducted also by Dr. Arthur Michaelson at the University of Cincinnati. He has found that under conditions of chronic ingestion of relatively low levels of lead from birth to adulthood, leading to increases in brain lead content and altered states of behavior, dopamine metabolism is either unchanged or partially slowed. Norepinephrine turnover is increased by as much as 30 percent and leaves the brain 14 percent more rapidly in lead-treated animals than in controls. Whether the increased turnover of norepinephrine is causative or associative in the experimental conditions under study is unknown. However, it is possible that increased motor activity and changes in brain catecholamine metabolism may correspond to early responses of lead exposure before overt signs of lead toxicity.

Behavioral effects. Dr. Michaelson has also studied the effects of chronic, low-level exposure to inorganic lead on the behavior of developing rats. The method of Golter and Michaelson for inducing lead intoxication was used in order to reduce the confounding effects of nutritional deficiency often associated with many of the earlier models of lead intoxication. Beginning at birth, the rat pups were incubated daily with 0.1 ml of 2 percent lead acetate, and at 15 days of age, the rats were weaned to a 400 ppm lead acetate diet and maintained on it for the duration of the studies. Such a paradigm produced rats chronically exposed to low levels of lead, but caused no differences in body growth, food and water intake, or appearance when compared to controls.

In tests of effects on activity, the data showed that the overall activity level of the control group was significantly higher than that of the lead-exposed rats. This relationship still existed during the second test period approximately four weeks later.

In studies of aggression, the data indicated that intra-species aggression (as determined by incidence of back bites) was increased in lead-treated animals compared to controls. Whether this was a species effect, a function of the type of aggression studied, or of age at testing requires additional research.

In addition to the studies reporting hyperactivity resulting from lead ingestion are those which show that hyperactive rats (like hyperactive childred) also respond paradoxically to an amphetamine challenge. Whereas amphetamine increases activity in normal individuals, it has a depressant effect on hyperactive children and lead-exposed rats.

Dr. Robert Rubin and his colleagues (Goldberg & Silbergeld) at Johns Hopkins University have shown that exposure of newborn mice to lead in the milk from mothers given lead in their drinking water results in a hyperactivity that persists for extended periods. The hyperactive mice exhibit no other overt symptoms of lead intoxication. Thus, this type of behavioral abnormality represents a very sensitive indicator of lead toxicity. As with hyperactivity in children (referred to frequently as "minimal brain dysfunction") this animal model of hyperactivity responds paradoxically to amphetamine and phenobarbital, the hyperactivity of the effected animals being decreased by the former and exacerbated by the latter. Treatment of hyperactive mice with a number of centrally acting drugs leads to the hypothesis that, at the neurochemical level, hyperactivity is an expression of an inbalance in both the cholinergic and catecholaminergic neurotransmitters. Thus, centrally active cholinergic and anticatecholaminergic drugs alleviate the hyperactivity while anticholinergic and catecholaminergic drugs exacerbate the hyperactivity. Brain minces of lead-hyperactive mice show a marked decrease in K^+- stimulated release of ACh while brain minces of mice treated with methylphenidate, a drug that reverses the hyperactivity, release control amounts of ACh. These results suggest a lead-induced block in the release of ACh as being a partial neurochemical explanation of the observed hyperactivity. These experiments represent a model system for the study of the relationship of minimal brain dysfunction and lead intoxication in children.

Dr. David Brown (University of Maryland) is attempting to determine the lead levels and stages of development of the rat at which the brain is sensitive to damage, using both biochemical and behavioral parameters. In studies of lead accumulation in nursing rats, a two-fold higher blood lead concentration was found in the 10-day-old pups than in 20-day-old pups, although both received the same doses of lead. The higher concentrations were associated with decreased renal and biliary secretion of lead in the younger rats. Unlike the case for the adult, the renal excretion of lead in the pup did not increase with increasing blood lead levels. These results suggest that the brain may be exposed to higher levels of lead in the 10-day-old than in the 20-day-old rat, producing more damage. Blood lead is also being measured in brain regions, but only moderate increases in brain lead levels in 20-day-old rats treated with lead from birth to 10 days have been found.

In behavioral studies in adults treated with lead as pups, it was found that the effect of lead on T-Maze performance was not increased by increasing the dose of lead administered. Thus the effect does not appear to be dose related at blood levels in excess of 100 ug/dl in the 10-day-old rat. More detailed studies of T-Maze performance have shown that the lead-treated rats tended to repeat nonrewarded behavior. Therefore, the rate of extinction of T-Maze performance was measured and found to be slower in the lead-treated group. No difference was found in locomotor activity measured in an actophotometer.

Renal effects. Though the major effects of lead appear to involve the nervous system, other effects have been documented also. Dr. Adrian Spitzer (Yeshiva University) has studied the effects of lead poisoning on kidney function. Renal blood flow (RBF), glomerular filtration rate (GRF), fractional excretion of sodium (FE_{Na}) and fractional reabsorption of phosphorous (TRP) were measured either two to three weeks after the onset of lead ingestion or two to three weeks after the end of a six week period of lead administration. The same variables were examined in a control group which were given tap water for drinking.

The results obtained so far indicate that an increase in GFR and in absolute reabsorption of phosphate occurs in rats which were submitted to a six week course of lead acetate ingestion. The tendency towards an increase in GFR and phosphate absorption was already manifested after two to three weeks of lead intoxication. RBF, FE_{Na}, and TRP do not appear to change.

Effects on lipid metabolism. Dr. Thomas Tornabene (Colorado State University) has compared the effects of lead on procaryotic and eucaryotic cells to develop information and reference sources on the molecular basis of the action of lead. Work with the bacterium *Micrococcus luteus* has revealed that Pb affects a biosynthetic parameter that is reflected in the biosynthesis of lipids, for which the cells apparently compensate genetically. There is a definite relationship between lipid contents of cellular membranes and lead without specific lipid-lead interactions. The nature of the interaction suggests that lipids have a role in the formation of inclusion bodies and the transport of lead.

Cadmium

Etiology and pathogenesis. Dr. Paul H. Weswig of Oregon State University and his co-workers have attempted to develop a clearer picture of body levels of cadmium relative to specific disorders, using the rat as a model. In accord with previous reports, they found that the blood pressure of male rats on a basal diet containing a small amount of Cd (150 ppm) increased gradually from about 115 mmHg to 150 mmHg after 16 weeks exposure. The blood pressure of females did not increase significantly. A genetically hypertensive strain of rats with usual pressure of about 160 mmHg showed a dangerous elevation to over 200 mmHg after 16 weeks on the diet. This suggests that individuals with preexisting hypertension may be put at substantial risk by Cd intake.

In these studies, renin and angiotensin-converting enzyme (ACE) were also determined. On addition of Cd to the basal diet, there was a rapid increase in renin from less than three units to about 15 units and then a leveling off to about twice normal. There was no effect on ACE with time. This shows a direct correlation between Cd intake and changes in pressure regulatory systems.

Dr. Donald Louria has studied the effects of Cd on certain enzymes. He has shown that cadmium interferes with alpha-1-antitrypsin in man. These effects

are dose related, reduction occurring in both alpha-1-antitrypsin levels and trypsin inhibitory capacity. No such reduction was found with other trace substances, including lead, zinc, nickel, iron and mercury. Heavy cigratte smokers (over 20 per day) showed a reduction ($p < .05$) compared to non-smokers in regard to trypsin inhibitory capacity. These studies may offer at least a partial explanation for the emphysema occurring in cadmium exposed industrial workers. The studies also suggest that heavy smokers may be more predisposed to pulmonary emphysema by virtue of reduction in alpha-1-antitrypsin activity (presumably related to the cadmium content of cigarettes). Plans are being made to analyze Cd workers for alpha-1-antitrypsin levels and to conduct more extensive studies of cigarette smokers.

Dr. Michael Wolcott at the University of Alabama has found that Cd affects the humoral immune system. The effects are detectable well before any overt signs of Cd toxicity. They are manifested as a large and rapid increase in the number of antibody secreting cells in the spleen and a large increase in light immunoglobulin chains in the blood and urine. The light chains apparently are produced in abnormal proportion to heavy chains by the altered spleen cells and presumably do not combine in the normal way with the heavy chains to form immunologically active antibodies. The implications of these events in the breakdown of resistance to infectious diseases are profound and are being investigated further.

The effects of Cd and other heavy metals on photosynthesis has been studied by Dr. Charles Miles (University of Missouri). Cadmium salts seem to irreversibly bind with the reaction center of photosystem II and to be very toxic at low concentrations. The binding of cadmium with the reaction center of chlorophyll takes place very effectively at a much lower concentration than those of lead salts. The data agree well with results of other studies on the uptake of trace metals by green algae. Large amounts of lead were found to be taken up by the cells, but the effect on photosynthesis was much less than that of Cd which was less readily absorbed.

Dr. Tom Miya (Purdue Research Foundation) has investigated the interaction between Cd and therapeutic agents and the possible mechanisms involved. Studies include effects of Cd on hepatic metabolism of drugs, on hexobarbital-induced sleep in hepatectomized rats, and on central nervous system sensitivity. The studies indicate that cadmium potentiates the duration of action of central nervous system depressants and suggest that the major underlying basis for potentiation of drug response is an inhibition of drug-metabolizing enzyme activity in the liver.

Cadmium-binding proteins. Dr. David Natusch (Colorado State University) has studied the interactions of various toxic metal species with biomolecules. The research deals primarily with (1) the chemistry of Cd-metallothionein and (2) factors affecting transport through phospholipid membranes.

Methodology for isolating substantial quantities ($\sim$ 500 mg) of metallothionein from horse kidney has been developed and supplies of this

protein are now available. Preparations were characterized in terms of trace metal content, chromatographic purity and n.m.r. spectra and have been shown to be reproducible between batches. Cadmium and zinc concentrations were found to be essentially constant with six metal ions per metallothionein monomer (MW $\sim$ 6600). Electrochemical and spectroscopic methods have been used to determine the stoichiometry of the metallothionein-metal complexes by titration. The electrochemical methods show that the stability constant for cadmium binding is $\sim 10^8$ times that for the zinc complex. The absolute stability constant and lability of cadmium-metallothionein are currently being determined. A lower limit of 10^{24} for the cadmium-metallothionein stability constant has been established. Proton magnetic resonance studies (220 M Hz, Fourier Transform) show that excellent high resolution spectra of both natural and apo-metallothionein can be obtained. The spectral differences suggest that further work will provide useful information on the ways in which cadmium binding modifies the structure of apo-metallothionein. Carbon^{13} magnetic resonance spectro of metallothionein have also been obtained. These show extensive peak broadening and measurements of relaxation times are being conducted to obtain information on molecular motions. Extensive efforts are being made to obtain cadmium^{113} Fourier Transform nuclear magnetic resonance spectra of metallothionein. Initial results have established that the binding situation of cadmium is unique and apparently unlike that of cadmium in other, apparently similar, complexes. This is in accord with the results of electrochemical experiments and of ESCA studies. It is apparent that both proton and cadmium magnetic resonance studies are intrinsically able to provide substantial information about the binding characteristics of cadmium. Initial results suggest that cadmium is probably bound to three sulfhydryl groups.

Dr. Robert Cousins (Rutgers University) has studied the metabolism and biochemical actions of cadmium. He has shown that the stimulation of protein synthesis following Cd injection is limited to Cd-binding protein. Cd deposition and the deleterious effects of the metal were enhanced by a low calcium diet.

In an effort to explain the increased uptake of Cd in rats fed low calcium diets, the uptake of ^{109}Cd was examined in rats fed these diets for short periods of time. The results show that rats fed adequate levels of calcium sequester Cd as a low molecular weight binding protein (CdBP) in the intestinal cells but those fed low levels of calcium do not sequester as much Cd with CdBP or other mucosal proteins. These results indicate CdBP prevents Cd transfer to the circulation at the serosal surface of the mucosal cell and in this way prevents absorption. To evaluate the function of this mucosal barrier, the metabolism of injected vs. oral ^{109}Cd was investigated in rats fed low or adequate calcium. The organ and tissue distribution of injected ^{109}Cd was identical in both groups. However, the uptake of oral ^{109}Cd was elevated 1.3 to 2.0 fold in the rats fed low calcium diets. These new data suggest that the intestine functions as a calcium-dependent barrier against ingested cadmium.

Other Metals and Combinations

Etiology and pathogenesis. Over many years, Dr. Henry Schroeder (Dartmouth Medical School) has been assessing the role of trace metals in disease. These studies have formed the basis of concern about a number of metals. The work is now in its terminal phase due to Dr. Schroeder's death.

Studies are nearing completion on the influence of the essential trace metals Cr, Mn, Co, Cu, Zn, and Mo on the retention of abnormal metals in tissues of rats. Analyses of heart, lung, liver, kidney, and spleen of rats given zirconium, niobium, germanium, vanadium, or tin for life were compared with these organs of rats not fed the metals to ascertain antagonisms or enhancements of metal depositions. The work is largely complete and is awaiting a few more analyses and statistical evaluation. The highest values for zinc were found in niobium-treated rats, indicating a heretofore unknown interaction between zinc and niobium.

Metal ion transport and interactions. Studies of metal-ion interactions with and transport through phospholipid membranes are nearing completion. Large quantities of chromatographically pure phosphatidyl choline are routinely isolated from egg yolks and characterized in terms of the light scattering and proton magnetic resonance properties of sonicated dispersions. Problems of variability between preparations have been overcome. It is now apparent that metal ions become electrostatically associated with the surface of phospholipid vesicles. Metal ions reside close to the negative phosphate group and the counter anion resides close to the positively charged $N(CH_3)_3+$ head group. The ions diffuse rapidly within the electrical double layer at the vesicle surface. Several methods have been developed for observing metal ion transport through vesicle membrane bilayers. These involve observation of the movement of paramagnetic ions (e.g., Cu^{++} and Mn^{++}) into the vesicle by proton magnetic resonance; observations of transport by the quenching of metallofluorometric indicators (Calcein Blue and 8-Hydroxyquinoline-5-sulfonic acid) as a metal ion moves into or out of the vesicles, and similar observations utilizing the metallochromic indicator, murexide.

Results of transport studies conducted at 39°C show that the passive transport rate depends markedly on the identity of both a metal ion and its counter anion. For example, the transport rate of $CuCl_2$ is extremely slow but that of Cu-acetate occurs within a few seconds. The counter anions sulfate, histidine, glycine, alanine and propionate exhibit intermediate behavior. The transport process is first order and the activation energy is of the order of 10-14 k cals/mole. These results indicate that passive transport of metal ions is regulated by the accompanying anion or ligand and that the actual transported species is probably singly charged. It appears, therefore, that the chemical form in which a given trace metal exists may be the determining factor in transport. An extensive theoretical model has been developed to describe this anion-mediated passive transport. Its predicted dependences of transport rate on concentration, temperature, and cation-anion pair are in accord with experimental findings.

Metal teratogenesis. Dr. Virgil Ferm of Dartmouth College has investigated the biological enigma of copper in embryogenesis. On the one hand, there is

evidence that copper deficiency is detrimental to embryonic survival in the rat and chicken. On the other, there is evidence that an excess of copper has a detrimental effect on implantation and blastocyst development. In an effort to define more precisely the action and role of copper in early embryonic development, Dr. Ferm treated hamsters on the eighth day of gestation with chelated and nonchelated copper compounds. He found an increase in the number of embryonic resorptions as well as the appearance of developmental malformations. Malformations of the heart appeared to be a specific result of copper. Copper in the chelated form of copper citrate was more embryopathic than that administered as copper sulfate.

In the same laboratory, Dr. Thomas Gale has explored the teratogenic effects of mercurials, lead, chromium, and ytterbium. With inorganic mercury, he found the major manifestations of embryonic damage included an increased resorption rate as well as an increased frequency of small, retarded and edematous embryos. Several other minor malformations were noted. Comparative studies on the teratogenicity of chromium and lead show that the metals have similar effects and that mixtures of the two are additive with respect to the degree of edema, embryo growth retardation, and tail abnormalities.

Also in progress is a project initiated last year on the embryotoxicity of chromium and lead. The internal defects induced by chromium have been found to be few in number except for a curious retardation of palate closure in embryos treated with the higher doses of chromium trioxide. A study of the skeletal system of chromium-treated embryos is now in progress.

A project was initiated this year to compare the incidence and types of external, internal and skeletal malformations of several metal chelate complexes (cadmium, lead, and mercury citrate) with the known teratogenicity of inorganic salts (cadmium sulfate, lead nitrate, and mercuric acetate). The data on external malformations suggest that the complexes are not as embryotoxic as the inorganic salts.

Metal chelation. A number of investigators are attempting to design agents useful for clearance of heavy metals from the body and therapy of intoxications. Dr. Roger Roeske (Indiana University Foundation) has examined several classes of possible heavy metal binding agents by use of a computer. Each class is based on naturally occurring compounds. The most interesting class of these so far has been the steroids. Relatively simple modifications of steroids would suffice to satisfy the ligand requirements of Hg^{++} and $CH_3\text{-}Hg^{+}$. Requirements for Pb^{++} usually involved one or more mercapto groups in close proximity to an oxygen or nitrogen atom for the donation of an electron pair.

Three simple mercapto steroids were synthesized: thiocholesterol (TC), 3β-mercaptocholanoic acid (MC), and 3α-mercapto-5α-androstan-17-one (MA). Each of these three was tested for the ability to remove Pb^{++} or Hg^{++} from a chloroform solution of diphenylthiocarbazone (dithizone) saturated with the heavy metal. All three mercapto steroids were equally good for Hg^{++}, but only MC was effective for lead. TC was moderately effective and MA not at all.

A study of the protective effects of the steroids in vivo on mice poisoned by lead or mercury has been initiated. TC was found to protect mice from Hg^{++},

CH_3-Hg^+, and to a lesser extent from Pb^{++}. An encouraging finding is that TC is effective when given orally.

Other interesting compounds, e.g., thioethers, are under investigation by Dr. Robert Neal and his colleagues at Vanderbilt University.

Dr. Arthur Aronson (Cornell University) has studied the mechanisms of toxicity of CaEDTA with the goal of finding ways to reduce its toxicity in treatment of heavy metal poisoning. He has found that massive doses of cortisol or dexamethasone (such as are used in the management of septic and traumatic shock in dogs) depress significantly the urinary excretion of hydroxyproline and antagonize the labilizing effect on lysosomes associated with CaEDTA administration. This suggests a possible avenue of exploration in the treatment of CaEDTA toxicity. Similarly, either Zn or Co chelates of EDTA protect against lysosomal labilization, suggesting the possible use of mixed chelates as a means of minimizing the toxic hazards of CaEDTA administration.

The association of CaEDTA administration with collagen degradation, increased lysosomal fragility, and the depressant and synergistic effect of lysosomal stabilizers and labilizers on urinary hydroxyproline excretion, opens new avenues for considering the interrelationships of metals in these phenomena.

Dr. Derek Hodgson (University of North Carolina) has continued his work on the mode of binding of toxic metals with chelating agents, with the ultimate goal of developing more efficient and less toxic therapeutic agents. Because of the tendency of cysteine per se to undergo oxidation and to be incorporated into protein, he has chosen to work with cysteine derivatives, most notably S-methylcysteine and penicillamine. He has prepared a variety of complexes of these ligands, including S-methylcysteine complexes of cobalt, platinum and palladium. The S-methylcysteine complexes are more stable than those of the parent amino acid and also form materials which are more crystalline and more tractable from a crystallographic viewpoint.

Cysteine and its derivatives are potentially tridentate, with potential binding sites at sulfur, nitrogen (amino) and oxygen (carboxyl). Other workers have recently shown by crystallography experiments that cysteine derivatives bind strongly to thallium, cadmium, mercury and molybdenum through only two of the three available sites. The crystal strucuture of the cobalt complex bis(S-methyl-L-cysteinato)cobalt(III) perchlorate monohydrate has been solved. It was found that the cysteine derivative is tridentate in this complex. The coordination about the cobalt atom is roughly octahedral, each substituted cysteine ligand being coordinated to the metal through S, N, and O, and each metal being bound to two such ligands. The S atoms occupy trans sites, and therefore, since the ligands are optically pure, the N and O atoms are necessarily cis. The Co-S, Co-N, and Co-O bond lengths of 2.261, 1.93 and 1.90 A, respectively are very similar to the sums of the respective covalent radii of the atoms of 2.26, 1.92, and 1.88, respectively. Hence, in this complex all of the binding sites of the ligand bind very tightly to the metal. This infers that S-methylcysteine should be an extremely efficient antidote for cobalt poisoning. It is noteworthy that, while

tridentate coordination by cysteine would appear to be highly favorable, this is the only reported example of an octahedral complex in which all six sites are occupied by donor atoms from two cysteine ligands.

Dr. Mark Jones (Vanderbilt University) has carried out chemical and animal studies on a new chelating polymer which binds mercury (II) very selectively and is also capable of providing very effective protection for mice against the action of CH_3HgCl. This polymer, when used as an antidote, is administered orally, and presumably interrupts the enterohepatic circulation of methylmercury derivatives. Chemical studies on the polymer show that the available donor sites react very rapidly with Hg^{2+} but interact only slightly or not at all with other metal ions. Techniques have been developed for the preparation and purification of the polymer to the point where a product with quite reproducible characteristics can be made at will. The characterization of the new chelates has shown that mercaptothions appear to bind selectively to Hg^{2+} in a large number of chelating agents. The selectivity found in derivatives of mercaptoacetic acid appears to be slightly enhanced in the analogous derivatives of mercaptopropionic acid. These chelating agents will form complexes with other metal ions, but the solution stability of the complexes is much lower than that found for the corresponding complexes of mercury (II).

Effects of metals on heme degradation. Dr. Attallah Kappas (Rockefeller University) has initiated important new studies concerned with the effects of heavy metals such as lead, iron, copper, cadmium, etc. on heme degradation. These studies evolved out of some exploratory experiments in which attempts to dissociate P-450 from heme oxidation to biliverdin were made. A variety of data, which did not conform with the prevailing view that P-450 is essential for heme catabolism, led to this line of investigation.

Briefly, heavy metals have a prowerful capacity to induce de novo synthesis of microsomal heme oxygenase, the liver enzyme which oxidizes heme to bilirubin. The oxidative process is analogous to drug oxidation in that it requires molecular O_2 and NADPH and is localized in microsomes. However, the reaction is not P-450 dependent. This indicates the existence in microsomes of a heretofore unrecognized enzyme complex for heme degradation distinct from that for drug oxidation. The developmental pattern of this enzyme complex is quite different from that for drug metabolizing enzymes and the effects of metals and other chemicals on this system are under intensive investigation.

An interesting observation resulting from the developmental study of this new enzyme complex is that in the immediate postnatal period, heme oxidation in liver becomes "induced" by some endogenous mechanism such that biliverdin and bilirubin production exceeds the normal by 5X. Neonatal jaundice, therefore, results not only from immaturity of the glucuronide-forming system in liver, but from a great increase above normal in bilirubin production as well. Chemical means of controlling heme oxygenase activity in the liver of neonates are being developed. This may lead to an efficient and simple treatment for neonatal jaundice, with its dangerous potential for neurologic damage.

The mechanism of metal induction of heme oxygenase is under study also. An SH-dependent repressor macromolecule is believed to be involved in controlling the structural gene coding for heme oxygenase. The enzyme reaction itself appears to be flavoprotein-dependent.

The significance of these new observations is that they define an entirely new and unsuspected effect of metals on the liver and provide a new mechanism through which metals can exert toxicity in the organism, since enhanced heme, and therefore P-450 degradation, would be followed by a wide series of biological consequences resulting from impaired metabolism of drugs, carcinogens and other environmental chemicals.

Effects of metals on infections. Dr. Donald Louria (New Jersey Medical School) has begun studies concerned with the influence of trace metals on experimental infections. He has shown that administration of lead to mice by mouth, subcutaneously and intraperitoneally augments the severity of Candida and Listeria infections. Lead loading reduced clearance of organisms from tissues and produced an increase in tissue titers relatively late in the course of the infection. These findings occur at various lead levels, including those comparable to blood concentrations found in man. Further studies have shown that lead does not impair phagocytosis or intracellular killing in human or mouse leukocytes. Lead-treated mice showed normal polymorphonuclear mobilization. Several studies suggested that lead interferes with the lymphocyte-macrophage system. When added to human peripheral leukocytes, it appears to interfere with migration inhibition factor. Lead also inhibits one hour (mononuclear) clearance of staphylococci from the mouse peritoneal cavity. Furthermore, lead loading appears to reduce the capacity of guinea pigs to respond to BCG. Additionally, if animals given BCG become sensitized to it, and subsequently are lead loaded, the skin response to tuberculin is thereafter reduced.

Pesticides

DDT and Related Compounds

Behavioral effects. Chronic effects of insecticides on mammalian behavior have been studied by Dr. James Yarbrough (Mississippi State University), using the old-field mouse, Peromyscus polionotus. While behavioral data were variable, there were fairly consistent changes in digging, grooming, escape attempts, and number of entries into maze arms in mice fed 500 ppm DDT in the diet. There was no apparent correlation of behavioral changes with dosage level. However, there was a relationship between behavior and duration of treatment.

Biochemical effects. Dr. Yarbrough also studied some physiological-biochemical effects of DDT. Treatment of mice for tests was identical to that of the behavioral testing except that they were randomly sacrificed on a weekly basis throughout the 28-day test period to obtain tissue for analysis.

Enzyme assays were performed on selected tissues sampled at 14 and 28 days of the dietary treatments. Microsomal oxidases were determined on liver tissue samples and soluble enzymes (LDH, GOT, and GPT) were performed on homogenized tissues of liver, brain and kidney. The microsomal enzymes in the livers of

DDT exposed mice were elevated and, in general, were increased with time of DDT exposure. That is, cytochrome P-450, cytochrome b_5, NADPH cytochrome c reductase, NADH cytochrome b_5 reductase, and NADH cytochrome c reductase, all showed significant increases when compared to controls and were increased in week four over week two.

The increases in hepatic microsomal oxidase enzymes represent induction by DDT. Induction is further illustrated when protein content of whole tissue homogenates and microsomal preparations are compared between the dietary DDT treatments. Based on a whole homogenate determination, liver protein decreased by about 35 percent. When microsomal protein was compared, there was up to a three-fold increase in protein over the control for the dietary DDT treatments.

There were some apparent changes in certain soluble tissue enzymes related to the dietary DDT treatments. The liver LDH level decreased by 56.6 percent for the 500 ppm DDT treatment at 28 days. This same pattern of decrease was seen in the LDH levels of brain and kidney. The liver GOT levels showed the same trend as the liver LDH levels, with a decrease at four weeks of 42.6 percent for 500 ppm DDT dietary treatment. However, GOT levels of brain and kidney remained essentially unchanged, as did GPT levels for all tissues.

The tissue levels of LDH, GOT, and GPT in P. polionotus were essentially comparable to tissue levels in the laboratory rat (Sprague-Dawley Strain). The liver LDH decreases (56.6 percent) with dietary DDT exposure were comparable to decreases observed in laboratory rats during three to four weeks of mirex feeding.

The proliferation of the smooth endoplasmic reticulum induced by DDT and many other compounds may be associated with changes in hepatic ribonuclease activity. A rapid decrease in the activity of this enzyme is observed prior to the increase in mixed function oxidase activity. The characteristics of the membrane and soluble enzyme in liver tissue are being established and their role in the DDT-induced proliferation of the membrane system will be explored. Even exposure to pine shavings used as animal bedding produced a pronounced decrease in the activity of the soluble enzyme, indicating the sensitivity of this system to external agents.

Maternal transfer of DDT. Maternal transfer of DDT to offspring via the placenta and lactation has been studied by dosing female rats orally with ^{14}C-ring-labeled p,p'-DDT. The half-life of DDT and its metabolites in milk was found to be 7.2 days and the maximum concentration of these components in the neonatal rats was observed from one to three days. The conversion of DDT to its metabolites was more pronounced in the adult liver than in the liver of the neonates. With pregnant females, DDT was readily absorbed and transported throughout the animal, and was deposited in the fetus. The half-life of the DDT in the tissues of the pregnant female ranged from 7.6 hours in the fetus to 16.1 hours in the kidney.

Metabolites of DDT and their actions. Dr. John Pritchard (University of South Carolina) has continued studies on the renal handling of DDT in the flounder

and the rat. Major emphasis was placed on the polar metabolite of DDT, DDA [2,2-bis (p-chlorophenyl) acetic acid], the predominant form excreted in the urine of most animals. It was hypothesized that since DDA is an organic acid, it should be eliminated via the same transport system which excretes phenol red and p-aminohippurate (the PSP-PAH system). If this were so, drugs, such as probenecid, which increases uric acid excretion via the PSP-PAH system might also hasten DDA excretion and, by mass action, DDT excretion.

He has now documented energy dependent accumulation of DDA in vitro by the renal tubules of two flounder species. In vitro uptake was inhibited by organic acids. Autoradiography showed highest accumulation of DDA in the cytoplasm of renal tubular cells, with the concentration in tubular lumen slightly lower. Renal clearance experiments in vivo demonstrated active secretory transport and reduction in net secretion by organic acids.

To evaluate the significance of such uptake to net DDA excretion, the renal clearances of DDA and a glomerular marker (inulin) were measured simultaneously in vivo in intact winter flounder. In order to assess DDA clearance accurately, plasma binding was determined. Binding was shown by ultrafiltration to be extensive (97 percent). Correcting for plasma binding, DDA clearance was 10-40 times the glomerular filtration rate. Thus, the energy dependent uptake seen in vitro was reflected in net active tubular secretion in vivo.

In experiments with rats, cortical slices were incubated in vitro in saline containing 10^{-5}M ^{14}C-DDA. Results obtained thus far are entirely consistent with those obtained for the flounder.

Dr. William Kinter (Mount Desert Island Biolaboratory) has been successful in pinpointing a Ca^{++}-activated ATPase in the shell gland mucosa of sensitive ducks as the long-sought site of DDE action. Regarding osmoregulatory failure, he has conclusively demonstrated that sensitivity of nasal gland Na,K-ATPase to DDE cannot account for recent kills of young sea birds.

Chlordane

Metabolism. In studies on the mechanism of insecticide degradation, Dr. Fumio Matsumura (University of Wisconsin) found at least seven metabolites of trans-chlordane in the feces of rats fed the insecticide orally. Of these, five were isolated in pure form. One was characterized as oxychlordane. The other four have not yet been identified. Feeding of cis-chlordane yielded four products identical to four from trans-chlordane plus two additional metabolites not formed from trans-chlordane. Efforts are being made to identify all metabolites.

Pentachlorophenol (PCP)

Transport mechanisms. Dr. Pavel Smejtek (Portland State University) is attempting to elucidate molecular mechanisms by which pesticides induce changes in transport characteristics of lipid membranes. He found that pentachlorophenol (PCP) increases membrane conductivity by a factor of 1,000 when present in aqueous phase at a concentration of 10^{-5} M/L. The pK of PCP is between 4.75 - 4.8, but the maximum of membrane conductivity dependence

on pH does not occur at the pK value of PCP as predicted by the dimer theory of Lea, Croghan, and Finkelstein. It depends instead on the type of lipids and the cholesterol content. In the presence of cholesterol in the membrane, the maximum is shifted by about 0.5 pH unit to the acid side. Cholesterol increases membrane conductivity for all lipid types tested.

No significant membrane potentials have been detected when there is a PCP concentration gradient across the membrane. The system generates membrane potentials only when a pH gradient is present. In the presence of PCP and of a pH gradient the membrane behaves as a "hydrogen-like" electrode. This is an indication that membrane permeability to PCP is greater than that of the adjacent aqueous unstirred layers. It appears that ionized PCP molecules are present in the membrane in large densities, presumably in the region of polar head groups. Partition coefficient measurements also suggest that neutral PCP is present in the membrane in considerably greater concentrations than those in the water phase. The preliminary value of the partition coefficient of PCP between aqueous and decane phases is about 1:2000.

The membrane conductivity in the presence of PCP increases with the cholesterol content in the case of PC membranes by a factor of about six. The dependence conductivity on cholesterol molar fraction is sigmoidal with the greatest slope in the vicinity of Y = 0.5. This result excludes the former proposition that cholesterol only decreases fluidity of hydrocarbon chains in the membrane core.

Toxaphene

Environmental impact. Dr. Kilgore et al. (University of California, Davis) is carrying out extensive studies on the environmental impact of chlorinated hydrocarbons. The single most important objective of the program is to determine and interrelate morphological and physiological hazards created by chlorinated hydrocarbons with their retention, distribution, and ultimate fate within the environment. This particular program project represents a concerted effort to assess both short- and long-term health-related problems resulting from the use of chlorinated hydrocarbons.

A part of the work is directed toward methods for reassessment of the registered uses of toxaphene in view of the potential ban on additional persistent organochlorine pesticides. An environmental concern which underlies toxaphene analysis is that one or a few components, or transformation products, may possess some undesirable property masked by the behavior of the remainder of the mixture. Thus, components of extreme persistence or high biomagnification potential, or those showing toxic effects to non-target organisms at low levels, are of particular concern.

In residue tests for toxaphene on tomato foilage, initial residues of ~100 ppm declined to ~20 ppm in 21 days. The primary dissipation mechanism is one of volatilization, since the 21-day residue was enriched in the less volatile components (seen clearly from capillary GLC runs) and was devoid of chemical breakdown products. In cotton fields treated with 8 lb/acre of toxaphene, initial leaf residues of 2,000 ppm declined to ~120 ppm after 61 days, and the same trend toward enrichment of less volatile components was observed.

Following harvesting of cotton from these fields, a residue of 48 ppm of toxaphene was found in seed cotton. Following ginning, a residue of 409 ppm was observed in the gin trash. The latter is particularly significant because a major problem exists in the San Joaquin Valley regarding ways of disposing of the sizeable "trash" which accumulates during ginning.

Toxaphene composition. Toxaphene consists of a poorly-defined mixture of at least 175 components. Most of these conform to the formulas $C_{10}H_{18-n}Cl_n$ and $C_{10}H_{16-n}Cl_n$ where n is 6 to 9. All the components previously identified (a total of five) were shown to possess the bornane ring structure, formed from rearrangement of camphene during chlorination. A new component has been isolated by column and gas-liquid chromatography, followed by crystallization. It was shown by x-ray crystallography to be 2,5,5-exo-8,8,9,10-heptachloro-dihydrocamphene. On examination of toxaphene by gas chromatography it is apparent that the major components are not represented by any of the standards examined. These still await identification.

A comparative toxicity study was undertaken on the pure dihydrocamphene derivative. The toxicities of pure dihydrocamphene and another previously identified bornane compound were compared using the housefly and mouse as test organisms. The mouse LD_{50} is twice that of toxaphene.

Investigations on toxaphene by Casida et al. have continued. Previous work has served to identify one toxic component as 2,2,5-endo,6-exo, 8,9,10-heptachlorobornane. The most toxic component has been identified as a mixture of 2,2,5-endo,6-exo-8,9,10-octachlorobornane and 2,2,5-endo,6-exo-8,9,10,10-octachlorobornane. These three materials account for the major protion of the toxicity of toxaphene to mice and goldfish and are likely to be the compounds of greatest toxicological and environmental concern. Continuing studies with ^{14}C-toxaphene further establish that it is rapidly metabolized and that the metabolites reside for only very short periods within the tissues of rats.

Two iron(II) porphyrin systems have been used to determine the mechanism of degradation to toxaphene; the microsome-NADPH and hematin-$Na_2S_2O_4$ systems under anaerobic conditions quickly degrade toxaphene and its toxic components to form a variety of products by processess that include dehydrochlorination and reductive dechlorination. It was found that the hematin-$Na_2S_2O_4$ system also degrades another chlorinated insecticide, Mirex, to form a series of reductive dechlorination products.

Parathion and Guthion

Reentry trials. Dr. Wendell Kilgore et al. (University of California, Davis) have continued studies to assess the public health hazard of parathion. In 1971 the U. S. Environmental Protection Agency banned the use of chlorinated hydrocarbons in compliance with the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act. Since then, those involved in pest management have turned increasingly to the use of organophosphorus pesticides to replace the less toxic but more persistent chlorinated hydrocarbons. This trend has resulted in a new class of occupational hazards for the agricultural

industry--pesticide residue poisoning, which is a result of prolonged contact with organophosphorus residues on treated crop foliage.

To protect agricultural field laborers, reentry interval (REI) standards have been adopted. The first reentry trial was completed this past year. The specific aims for this trial were (1) to measure the physiological effects of organophosphate residue exposure in a normal agricultural field labor population during routine hand-thinning operations in a peach orchard treated with guthion. The principal physiological effects monitored were whole blood ChE activity, reflex strength, and urinary para-nitrophenol excretion; (2) to correlate ChE activity with guthion residue levels on the foliage; (3) to field test earlobe puncture as a method of obtaining blood samples from agricultural field laborers; (4) to compare the reliability, validity, and practicability of the leading ChE assay methods: a colorimeteric micromethod, a pH-stat macromethod, and a pH-stat micromethod; (5) to monitor meteorological and human behavioral factors that may influence ChE activity; and (6) to identify and define the major operational problems in the implementation of a reentry trial conducted to meet the above objectives. The principle which governed the design and implementation of this reentry trial was adherence to usual agricultural practices. The development of reliable REI's is dependent upon the extrapolation of reentry trial results to the general agricultural environment. A comparison of the farmworker's baseline and test values of whole blood ChE activity indicated that no appreciable change in enzyme activity occurred as a result of exposure to guthion residues after the expiration of a 14-day reentry interval.

Earlobe puncture as a method of obtaining blood samples was found to be unacceptable due to participant apprehension, the necessity of making a slit at least 10 mm deep and 5 mm wide (which may reopen as long as eight hours after puncture), and the need for two technicians to perform the procedure (which can take five to ten times longer than venipuncture). A control group in all future reentry trials is felt to be mandatory in order to obtain reliable reentry interval data.

In experiments to assess climatology in relation to pesticide application the data on weather factors, application parameters, air sampling, and fallout is incomplete at this date. Rough data indicate significant levels of airborne Guthion found during the treatment and surprisingly high levels during the worker reentry period. In these tests, nearby upwind orchards were being treated with Guthion while workers were in the test plot and air sampling was in progress. Thus, existing air data may well be contaminated by airborne Guthion from the nearby applications. It is evident that there were airborne and fallout levels of Guthion that could be detrimental to the health of applicators and reentering workers. Further field tests of a similar nature will be conducted in the 1976 and subsequent seasons.

Factors in parathion toxicity. Dr. Sheldon Murphy and his colleagues (Harvard University) have studied the influence of age on toxicity and metabolism of parathion and methyl parathion in rats. For rats of five ages studied (1, 12-13, 23-24, 35-40, and 56-63 days), there was a gradual decrease in susceptibility to poisoning by both insecticides with increasing age up to 35-40 days for both sexes.

Age differences in susceptibility were not related to differences in sensitivity of cholinesterase to inhibition by paraoxon or methyl paraoxon in vitro. Oxidative formation of the oxygen analogs, oxidative aryl cleavage, and glutathione-dependent dealkylation and dearylation of methyl parathion and parathion were assayed in liver homogenates of male and female rats of the five ages. Rates of enzymatic detoxification of their corresponding oxygen analogs by A-esterase, glutathione-S-aryl- and S-alkyl-transferase and inactivation by binding were also investigated. In general, changes in LD_{50}'s with age for methyl parathion correlated better with changes in rates of reactions which represented detoxification pathways for methyl paraoxon and paraoxon than for reactions which represented direct metabolism of the parent insecticides. Both male and female rats became much less sensitive to the acute lethal effects of methyl paraoxon and paraoxon with increasing age. This is consistent with a hypothesis that changes in LD_{50}'s of methyl parathion and parathion with age are due to changes in rates of metabolism of the oxygen analogs.

Potentiation of OP activity. Organophosphate insecticides which have relatively weak anti-acetylcholinesterase (AChE) action and strong anti-carboxylesterase (CE) action strongly potentiate the toxicity of some organophosphorus insecticides which contain carboxyester groups, such as malathion. However, certain pairs of organophosphates which do not contain carboxyester groups are synergistic, and it has been proposed that they compete for noncritical sites of loss, e.g., CE. Recent research has been directed at examining the relationships between potentiation of OP insecticide toxicity and CE-inhibiting potency of bis-dinitrophenyl phosphate (BNPP), triorthotolyl phosphate (TOTP) and the defoliant SSS-tributyl phosphorotrithioate (DEF). These compounds are all 10 or more times as potent as CE inhibitors than as AChE inhibitors. Male mice were pretreated i.p. with TOTP, BNPP, and DEF at maximum CE-inhibiting doses which did not affect AChE, and subsequently challenged with varying doses of malathion, parathion, Guthion (azinphosmethyl), parathion-methyl or their respective oxygen analogs. The ratio of the dose of the insecticide required to produce 50 percent inhibition of brain AChE in control animals to the dose for this effect in pretreated animals was taken as the degree of potentiation. Maximum degrees of potentiation observed for both malathion and malaoxon were 15-20 after TOTP, 25-35 after BNPP and 10-12 after DEF. For parathion and methyl parathion and their oxygen analogs, the degree of potentiation by the three pretreatment compounds never exceeded three. For Guthion the degrees of potentiation were 6, 14, and 17 after BNPP, TOTP, and DEF respectively; while for its oxygen analog they were only 3, 6, and 4 respectively. Since parathion, methyl parathion, and Guthion did not contain carboxyester groups, the marked potentiation of Guthion was unexpected and appeared not to be related to its anti-CE activity since it was weakest of the compounds tested in this respect.

Effects of OP on reproduction and development. Dr. Raymond Harbison (Vanderbilt University) has continued studies on effects of organophosphates on development. He has found that trimethylphosphate, a potential environmental contaminant, produces sterility in male mice, rats, and rabbits. This effect is reversible but can be maintained by chronic treatment. In addition to sterility, muscular weakness is produced. Muscular weakness is also reversible but can be maintained by chronic treatment. The mechanism for

both effects appears to be inhibition of the enzyme choline acetyltransferase, which reduces synthesis of acetylcholine and renders epididymal spermatozoa infertile, probably by interfering with their motility. Also, inhibition of choline acetylase of brain and muscle depresses functional levels of acetylcholine and renders muscle tissue non-functional.

A developmental pattern of choline acetyltransferase activity was observed in spermatozoa. Spermatozoan choline acetyltransferase activity increases as it traverses the epididymis. Highest activity in both rabbit and rat was observed in the epididymal cauda or tail segments. The stimulus for this is unknown. Semen containing specific substrates, cofactors, or activating factors may initiate the development of choline acetyltransferase activity. In all species studied, the most mature sperm contained the highest choline acetyltransferase activity.

Sperm choline acetyltransferase activity exists in all three of the species studied--rat, rabbit, and man. Rat sperm choline acetyltransferase activity was higher than choline acetyltransferase activity of rabbit and man. Choline acetyltransferase activity of rabbit and man was comparable.

This is the first report of choline acetyltransferase activity in mammalian sperm. A complete cholinergic system, choline acetyltransferase-acetylcholine-acetylcholinesterase, exists in mammalian sperm. It appears that sperm motility is regulated by the nascent levels of acetylcholine. The rate limiting determinant is probably synthesis by choline acetyltransferase since temporary sterility can be produced by disruption of this system.

In general, newborn rats are more susceptible to organophosphorus pesticides than to chlorinated hydrocarbons. Two factors appear to be responsible for this. First both plasma and brain cholinesterase levels are low in the newborn, which predisposes the newborn to parathion-induced toxicity. Since cholinesterase levels are low, smaller amounts of paraoxon are required to inhibit a critical amount of cholinesterase to induce a toxic reaction. Second, the ability of the newborn to detoxify paraoxon is low compared to the adult. Development of the metabolic detoxication mechanism occurs during the first few weeks of postnatal life. Detoxification is stimulated by phenobarbital treatment and phenobarbital can protect the newborn against parathion-induced toxicity.

Dr. Barry Wilson (University of California), in studies on the effects of organophosphates on muscle and nerve cell growth and metabolism, has shown that the AChE of cultured muscle rapidly recovers from paraoxon, malaoxon, or diisopropylfluorophosphate (DFP) by synthesis of new protein, suggesting that rapid return of AChE after organophosphate poisoning is a general response of the cells. Return of AChE after DFP treatment in organ culture suggests such recovery is not merely a special effect of cell culture.

Primary cultures of nerve rapidly recover their AChE after DFP treatment. Since both nerve and muscle release AChE, there is a question as to which cell type(s) contributes AChE to the neuromuscular junction. Embryo muscle AChE (and AChE in denervated muscle) is at least partially regulated by

contractile activity; whether excitation of the muscle membrane alone is sufficient is unknown. Increase in AChE by ACh and analogs is not an induction--i.e., it is not directly dependent on DNA-mediated RNA synthesis. It seems to involve ACh-receptor and contractile activity.

Metabolism of OP. Dr. Robert Neal (Vanderbilt University) has continued to examine the mechanisms and consequences of the release and covalent binding of the thiono-sulfur atom of parathion. Studies using rat liver microsomes have shown that the sulfur atom binds to the macromolecules of the microsomal membrane. The maximal binding requires the presence of NADPH, is increased upon treatment with phenobarbital and 3-methylcholanthrene, and is inhibited by carbon monoxide. The majority, if not all, of the sulfur bound is in a form free of the remainder of the parathion molecule. The evidence indicates that the sulfur bound is that released in the mixed function oxidase catalyzed metabolism of parathion to paraoxon. The binding decreases the concentration of cytochrome P-450 in the microsomes and inhibits slightly the rate of the mixed function oxidase catalyzed metabolism of benzphetamine.

Studies on rat lung and brain indicate a mixed function oxidase enzyme system capable of metabolizing parathion to paraoxon and to diethyl phosphorothioic acid is present in lung and brain. In contrast to liver, the metabolism of parathion by rat lung and brain microsomes is not inducible by pretreatment of the animals with phenobarbital or 3-methylcholanthrene. The metabolism of parathion by lung microsomes is stimulated by NADPH and oxygen and is inhibited by carbon monoxide, anaerobic conditions, SKF-525A and piperonyl butoxide.

Other thiono-sulfur containing compounds were studied to see if the mixed function oxidase catalyzed release of covalent bonding of sulfur was unique to the phosphorothionate triesters such as parathion. One compound examined is the important industrial compound carbon disulfide. In these studies carbon disulfide (CS_2) was initially examined as a substrate for the hepatic mixed function oxidase enzyme system. These studies revealed that carbonyl sulfide (COS) was formed when carbon disulfide was incubated with rat liver microsomes in the presence but not the absence of NADPH. The formation of COS is inhibited when the reaction is carried out in an atmosphere containing carbon monoxide. The other product of the reaction leading to COS formation appears to be a highly reactive form of sulfur which covalently binds to the microsomal membrane.

Additional studies have indicated that the decrease in the activity of the hepatic mixed function oxidase enzyme system and the concentration of cytochrome P-450 seen on incubation of CS_2 with rat liver microsomes is the result of the binding of the sulfur atom released in the mixed function oxidase catalyzed metabolism of CS_2 to COS. Moreover, it appears that COS is further metabolized by the mixed function oxidase enzyme system to CO_2 and that, analogous to the metabolism of CS_2 to COS, the sulfur atom released in this reaction also binds to the microsomes and inhibits benzphetamine metabolism and decreases the concentration of cytochrome P-450 detectable as its carbon monoxide complex. The results of these studies also suggest

that the decrease in the concentration of cytochrome P-450 and the liver damage seen on in vivo administration of CS_2 to phenobarbital pretreated rats is due to the mixed function oxidase catalyzed released and binding of the sulfur atoms of CS_2. This does not appear to be the result of destruction of the heme group or its dissociation from the apoenzyme.

In addition to CS_2, nine thiono-sulfur containing compounds of varying structure (thioacetamide, thiourea, ethylene thiourea, propylthiouracil, methimazole, diethyldithiocarbamate, disulfiram, methylthiouracil, thiouracil) were administered by intraperitoneal injection to untreated, phenobarbital-pretreated and 3-methylcholanthrene-pretreated adult male rats. Seven of the eight compounds examined significantly decreased the concentration of cytochrome P-450 in the microsomes from each treatment group. Similar reductions in benzphetamine metabolism were also seen. When these same compounds were incubated with microsomes in the absence of NADPH, no significant reduction of cytochrome P-450 or benzphetamine metabolism was seen. When the oxygen analogs of six of the thiono-sulfur compounds were administered in vivo or incubated with hepatic microsomes either in the presence or absence of NADPH, there was no significant reduction of cytochrome P-450 or benzphetamine metabolism.

Pyrethroids

Structure-activity relations. Dr. John Casida et al. (University of California, Berkeley) have continued studies on pyrethroids. Pyrethroids have been considered to be highly effective and very safe insecticides but of potentially very limited use because of their rapid photodecomposition. This concept of their limitations has greatly changed in the past two years due to the development of extremely effective photostable compounds. Pyrethroids are now the logical candidates to replace DDT, the chlorinated cyclodienes, and other contact insecticides that can no longer be used because of tumorigenic or other unfavorable properties. The metabolism and photodecomposition studies carried out under this Program Project were important in making these developments possible.

The most effective pyrethroids are permethrin and a large series of related esters consisting of phenoxybenzyl or α-cyanophenoxybenzyl alcohol esterified with acids containing dihalovinyl or chlorophenyl substituents. With an emphasis on permethrin, the insecticidal (1R, trans)- and (1R, cis)-isomers with ^{14}C in the acid and alcohol moieties have been prepared and found to be rapidly metabolized in rats by oxidation of either the cis- or trans-methyl group of the dimethylcyclopropane moiety, by hydroxylation of the phenyl group, by cleavage of the ester, and by conjugation of the alcohol and carboxylic acid sites. There are also two very susceptible insect species that rapidly metabolize these two isomers--houseflies and cabbage loopers. Other continuing studies on the structure-biodegradability relationships of pyrethroids have shown that, in the mouse liver microsome-NADPH system, sites in the acid and alcohol moieties and the stereochemistry of the molecule are important in determining the relative rates and extents of esteratic and oxidative detoxification.

Piperonyl Butoxide

Previous experiments by Dr. Murphy et al. have shown that piperonyl butoxide (PB) reduced the acute toxicity of methyl parathion by 40X. The same treatment increased the toxicity of parathion by 2X. Thus a study was undertaken to determine if PB interacts in the same way with dimethyl (DMP) and diethyl (DEP) phosphorothionates. The findings suggest that although PB elevation of plasma levels of both DMPs and DEPs provides a potential for greater total oxon formation, a decreased rate of oxon production allows more time for demethylation of DMPs. This plus rapid reactivation of inhibited nerve tissue ChE appears to provide protection against DMP toxicity by PB. In the case of DEPs, inhibition of oxidative detoxification in the absence of an alternative dealkylation pathway, slow reactivation of ChE occurs.

Dr. Marvin Friedman (Medical College of Virginia) has studied the effects of piperonyl butoxide (PB) on mixed function oxidases and on metabolism of certain carcinogens. He found that PB inhibited phenobarbital induction of mouse liver aminopyrine demethylase, a type I enzyme, and aniline hydroxylase, a type II enzyme.

PB had a completely different effect on the induction of aryl hydrocarbon hydroxylase (AHH). In Swiss mice, PB, in combination with phenobarbital, caused a superinduction of AHH activity, which was considerably greater than with either compound alone. Similar observations were made with phenobarbital induction in BALB/C mice, where PB also synergized AHH induction by 3-methylcholanthrene. In these studies induction of aminopyrine demethylase was suppressed by PB.

In studies on the effects of PB on nuclear RNA synthesis, mice treated with PB and phenobarbital had nuclear RNA/DNA ratios of 0.75 while all controls were less than 0.5 and naive controls were 0.32. Therefore, the inhibitory effects of PB on enzyme induction do not appear to involve the genetic components of this process while the synergy in AHH induction does appear to involve genetic components. It appears clear that even though DMN demethylase activity is decreased, there is no alteration in nucleic acid or protein alkylation. This implies that alkylation is not a result of mixed function oxidase metabolism. It also appears clear that genetic controls of AHH induction are different from those of other mixed function oxidase systems.

The Molting Hormone, Ecdysone

Dr. John Casida et al. (University of California, Berkeley) have continued a study of the role of the molting hormone, ecdysone, in the regulation of the microsomal oxidases of the housefly. Alpha and beta ecdysone were compared with five ecdysone analogs as inducers of the enzymes. The enzyme assay results were tabulated against previously published data on the biological activity of the five analogs. An interesting correlation between the two kinds of data was that the compounds with the greatest biological activity, i.e., inhibition of development, were those with the greatest activity as inducers. An additional result was the discovery of another enzyme which

metabolizes these steroids. This enzyme is found in the soluble fraction of housefly homogenates and does not require any additional cofactors such as NADPH. The reaction is thought to be one of dehydrogenation at the C-3 position of ecdysone to produce 3-dehydro ecdysone. Furthermore, if NADPH is added to the system, the dehydrogenation product is further metabolized to a second product, which is assumed to be the 3-epimer of ecdysone.

The experimental insecticide, TH-6040 is known to inhibit chitin synthesis in insects. Techniques have been developed for studying ecdysone metabolism by the soluble enzyme. This permits study of the biochemical effects of the compound. Two kinds of effects were observed--a 4 to 12-fold stimulation of the activity of microsomal oxidases and a 50 percent inhibition of the ecdysone metabolizing enzymes. Both of these effects occurred two days after housefly larvae were placed on a diet containing the growth regulator. There was a direct correlation between the extent of inhibition of the ecdysone metabolizing enzyme and the biological activity of the compound. The study suggests that TH-6040 disrupts chitin synthesis because of a primary effect on ecdysone metabolism.

Organotin Compounds

Dr. John Casida et al. have continued studies on organotin compounds. Triorganotins continue to expand in numbers and amounts used as industrial biocides, miticides and fungicides. The microsomal oxidation of tributyltin derivatives has been clarified. Oxidation occurs at each carbon of the butyl group to yield α-hydroxybutyldibutyltin, β-hydroxybutyldibutyltin, γ-hydroxybutyldibutyltin, and δ-hydroxbutyldibutyltin derivatives. The α-hydroxy compound spontaneously degrades under physiological conditions to dibutyltin and butanol. The β-hydroxy compound, although more stable, degrades in enzyme systems and in living mice to yield dibutyltin and butene. With the γ-hydroxy compound, further oxidation occurs to produce the corresponding ketone. The δ-hydroxy compound is more stable than the other three, but it appears in only small amounts.

Considerable progress has been made in synthesis of hydroxycyclohexyltriphenyltin and hydroxycyclohexyldiphenyltin compounds. The studies are designed to lay the necessary background for metabolism investigations on tricyclohexyltin hydroxide (Plictran miticide). It is possible that Plictran forms many isomeric hydroxycyclohexyldicyclohexyltin metabolites. The antifeeding activity of triphenyltin derivatives with Tribolium confusum larvae was found to be attributable to the reduced activity of digestive enzymes, with the result that food is not digested and feeding stops. However, with mice, feeding studies establish that the toxicity of dietary organotins is not due to inhibition of digestive enzymes. The most sensitive indices of organotin toxicity found in mice appear to be reduced weight gain, loss in spleen weight, and alteration in blood erythrocyte and leucocyte levels.

Herbicides

Mode of action. Several herbicides have been found by Moreland et al. (North Carolina State University) to interfere with the cyanide-insensitive respiration and the oxidation of exogenous NADH in plant mitochondria.

However, the relation of these pathways to the cytochrome chain in plant mitochondria has not been fully elucidated previously. Evidence was obtained this past year that the cyanide-insensitive pathway branches from the cytochrome pathway prior to the b cytochromes. Electrons from exogenous NADH were found to enter the cytochrome chain at, or just prior to, complex III.

Metabolism. In the studies of thiocarbamate herbicides, which are known to be biodegradable in plants and mammals, the mechanism of degradation and their mode of action are incompletely understood. Further studies, however, support the previous hypothesis that the primary pathway of metabolism with many thiocarbamates involves sulfoxidation by the liver microsome-NADPH system and then cleavage of the sulfoxide by the glutathione S-transferase system of the liver-soluble fraction. The thiocarbamate sulfoxides are excellent carbamoylating agents for such compounds as coenzyme A, glutathione and N-acetylcysteine. Rats administered thiocarbamates and thiocarbamate sulfoxides excrete the corresponding S-(N,N-dialkylcarbamyl)-mercapturic acids, establishing the in vivo relevance of this reaction sequence. Other research on thiocarbamates centers on their use for weed control in corn, where crop injury often limits the effectiveness. This injury can be minimized by the addition of small amounts of certain dichloroacetamides to the thiocarbamates. Interestingly, this antidotal action was found to be attributable to a very unusual phenomenon; the antidotes stimulate corn roots to synthesize increased levels of glutathione and glutathione S-transferase enzyme. This combination then effectively detoxifies the thiocarbamate sulfoxides to prevent injury to the corn. Two other studies of especial note are on (1) the benzoylphenylurea insecticides--which indicate that in mosquito larvae, as previously noted with housefly larvae, these compounds block chitin synthesis and increase the phenoloxidase activity, and (2) the N-nitrosamines--where electrophilic cleavage reactions in the gas phase may have relevance to the structure-activity relationships of these compounds as carcinogens.

Miscellaneous Studies on Pesticides

Dr. Frank Guthrie and his colleagues (North Carolina State University) continued their studies on pesticide toxicity mechanisms. Studies of cytochrome P-450 and microsomal mixed function oxidation have been continued with a variety of environmental chemicals and a number of different organisms. The studies include development of new methods for SDS-polyacrylamide gel analysis of microsomal proteins and the characterization of qualitatively different forms of cytochrome P-450 separated by differential centrifugation. Investigation of the biochemical genetics of resistance to insecticides showed that the gene responsible for type I ligand binding to cytochrome P-450 is probably the most important in oxidative resistance and can be separated from other genes affecting this cytochrome by crossing over. Two new series of methylenedioxy compounds have been synthesized and their interactions with hepatic cytochrome P-450 from mice and rabbits is being tested in an attempt to further elucidate the interactions of methylenedioxyphenyl synergists.

In studies on the molecular basis of toxicity, emphasis has been in two areas (1) the purification of glutathione S-transferases from mouse liver and

from resistant houseflies and (2) a survey of glutathione dependent reactions in susceptible and resistant houseflies.

A glutathione transferase involved in the conjugation of atrazine has been purified approximately 50-fold with a 25 percent yield from mouse liver. Sufficient enzyme has been obtained to evaluate some of its properties as well as to conduct a specificity study with various pesticides.

A glutathione transferase from diazinon-resistant houseflies has been partially purified 40-fold using dichloronitrobenzene as the substrate. Analytical electrophoresis showed nine protein bands of which two have activity. Preparative electrophoresis is in progress, hopefully as the last step in purification.

A survey was conducted on glutathione-dependent reactions in five resistant and susceptible housefly strains. The substrate utilized in the assays were methyl iodide, dichloronitrobenzene, γ-BHC, DDT, parathion and diazinon. A parallelism was found for the following reactions in the various strains: methyl iodide conjugation, dichloronitrobenzene conjugation, O-deethylation of parathion and diazinon and γ-BHC conjugation. The conjugation of the p-nitrophenyl and pyrimindinyl moiety of parathion and diazinon respectively was mediated by two different enzymes. Also the glutathione-dependent reaction with DDT involved still another enzyme system.

Studies on distribution of pesticides by blood proteins were expanded to a determination of binding constants of the heavy lipoprotein fraction found to be of greatest importance. Utilizing the Scatchard equation, the binding constants for three insecticides in two organisms were determined. The possible competition between binding of insecticides and sterols has been initiated. In other experiments, OP and carbamate insecticides were found to inhibit the ability of mice to thermoregulate whereas DDT had no action on temperature control.

A subproject concerning hazards of reentry into pesticide-treated fields involved studies to determine reentry intervals for monocrotophos involving human volunteers. Although rain interferred with these studies, it was found that the present EPA standard of 48 hours is not adequate to protect agricultural workers, and a reentry interval of 72 hours seems more tenable.

Cholinesterase of high purity is necessary to further advances in the kinetics of this target enzyme and its inhibition by various insecticides. Although an enzyme of high purity has been attained, alterations of present methods have advanced this method considerably during the past year. A major innovation was substitution of a pH 4 for a pH 3 step at the first column step which had a significant effect on a contaminant. Further characterization of the horse serum enzyme confirms a molecular weight of 100,000, a trimer with three active sites per molecule.

Another major effort in this subproject concerned characterization of rabbit serum cholinesterase. It was found that several cholinesterases are present in rabbit serum, one of which acts more like an acetyl than a butrylcholinesterase.

With organophosphorus (OP) and methylcarbamate (MC) compounds, most of the toxic effects are attributable to cholinesterase inhibition. Current studies are concentrating on those compounds or systems in which this is not the case. One of these studies is on the high toxicity of certain bicyclic OP compounds which rapidly act on the central nervous system to produce violent convulsive seizures. In structure-toxicity studies with 65 small cage compounds, the structural requirements for high mouse toxicity have been delineated and it has been shown that the phosphorus atom is not an essential feature for this action.

In continuing studies on the mechanism by which a great variety of OP and MC compounds induce embryonic abnormalities in chicken embryos, a major aspect of the phenomenon has been clarified. The teratogenic compounds diminish the normal embryo NAD levels to as low as 10 percent of normal. In fact, there is a direct association between the NAD levels and the teratogenic signs, and alleviating agents for the teratogenesis elevate the NAD to near normal levels. The relevance of these findings to mammals and to other actions of OP and MC compounds now needs to be evaluated.

In other research on OP compounds, progress is continuing on a definition of the metabolites and photoproducts of cis- and trans-dioxathion--major components of an important miticide. At this point, the mono- and dioxon derivatives appear to be of greatest toxicological interest. In a final study on MC compounds and some of their metabolites, it was established that chemical ionization-mass spectrometry provides important analytical information not obtained with the electron impact technique.

Dr. Leon Terriere (Oregon State University) has continued studies on the mechanisms by which insects metabolize foreign compounds. He has been able to solubilize housefly microsomes and to resolve the solution into two cytochrome P-450 fractions. The relative amount of P-450 in the two fractions varies depending on whether susceptible or resistant houseflies are used in the preparation of the microsomes. These experiments suggest that the housefly utilizes at least two forms of cytochrome P-450 and that one of the forms is greater in resistant strains. The microsomal enzymes isolated from fleshflies and blowflies were similar in properties to those studied in the housefly.

Studies were conducted by Dr. Donald Crosby (University of California, Davis) to determine the metabolic capabilities of lower aquatic species with respect to organochlorine, carbamate and organophosphate insecticides. Of the three phyla examined, the higher phylogenetic order, echinodermata, was best equipped to carry out metabolic transformations. Mollusca exhibited variability with the mussel, a bivalve, showing limited ability to utilize established pathways while the snail, a gastropod, metabolized foreign compounds almost on a level with the echinoderm. The coelenterate, sea anemone, similarly to the mussel, showed limited metabolic ability with these compounds. While oxidation and hydrolysis reactions were readily apparent for parathion, particularly in the starfish and snail, hydrolysis of the carbamate group in carbaryl was not. Absent also was the ability to conjugate p-nitrophenol and to reduce the nitro group of parathion or trifluralin.

Dr. Paul Dahm (Iowa State University) has studied the uptake of turbufos in soil, field corn, sweet corn, pop corn, and soil from Iowa fields. Residues of terbufos, expressed as the fully oxidized sulfone (O,O-diethyl S-(tert- butylsulfonyl)methyl phosphorothiolate), in field-corn forage ranged from a high of 0.30 ppm 40 days posttreatment (four pounds furrow application) to a low of 0 ppm 60 days postreatment (one pound band application). No detectable residues were found in field corn grain plus cob harvested for silage (100 days posttreatment) and quantities in stover of the same maturity showed only 0.01-0.03 ppm. Analyses of fully mature grain (160 days) showed no residues. Quantities of terbufos in soil where field corn was grown ranged from 55 ppm immediately after application (four pounds furrow application) to 0.2 ppm 160 days posttreatment (one pound band application). Sweet corn grain and pop corn grain sampled at edible maturity showed no residues of terbufos even though 7 to 10 ppm were still in the accompanying soil.

Laboratory tests were performed to determine the oxidation of terbufos in three Iowa surface soils (Clarion, Harps, and Webster). Soil samples were incubated at 22 $\pm$ 1°C for 0, 1, 3, 7, 14, and 21 days. Terbufos was rapidly oxidized to its sulfoxide. The parent compound had an approximate half-life of four to five days. Terbufos sulfoxide reached a maximum concentration after two weeks of incubation, and terbufos sulfone did not appear until one week after the incubation was started. Significant differences were found in the ability of the three soils to oxidize terbufos. The sulfoxide and sulfone had no inhibitory influence on soil microbial activities.

Degradation of Aromatic Compounds by Microorganisms

Dr. Stanley Dagley has continued his tudies on the enzymatic degradation of aromatic compounds by microorganisms. A novel pathway for degrading protocatechuate in *Bacillus* spp. to malic acid has been found. Meta fission of protocatechuate takes place between C-2 and C-3 of the nucleus, and not between C-4 and C-5 as in previously studied species. This shows that the point of ring fission cannot be determined simply by the chemical properties of substituent groups (carboxyl in this case).

Further studies on specific degradative enzymes reveal that gentisate 1,2-dioxygenase has a molecular weight of 154,000 and appears to consist of four subunits of identical size. It is Fe^{2+}-activated and attacks a range of halogen- and alkyl-substituted gentisic acids. In *Moraxella* species the degradation of these compounds is limited by the substrate specificity of its GSH-dependent maleylpyruvate isomerase, and not by gentisate 1,2-dioxygenase.

The studies indicate that an organism may be able to elaborate two separate suites of enzymes, each of which is capable, in principle, of degrading one particular growth substrate. Thus, 4-hydroxyphenylacetic acid (HPA) is catabolized via the homogentisate pathway in *P. acidovorans* and via homoprotocatechuate in *P. putida*. However, all the enzymes for degrading homoprotocatechuate can be induced in *P. acidovorans*. The pathway is not available for HPA catabolism because this species does not possess a

3-hydroxylase. In addition, P. putida, while catabolizing protocatechuate by ortho fission, elaborates a dioxygenase specific for gallic acid when exposed to that compound and then converts the ring-fission product of gallate to pyruvate by meta fission reactions. It appears that any one organism may possess a variety of catabolic routes available for compounds having closely related structures and that one pathway is chosen, in preference to the rest, for catabolizing a particular growth substrate.

Similar studies are being conducted by Dr. David Gibson (University of Texas). He has reported isolation of a toluene dioxygenase enzyme system from Pseudomonas putida. He has resolved the system into three protein components each of which is required for enzymatic activity. Two of the proteins, a non-heme iron protein and a flavoprotein, were purified to homogeneity.

Previous studies have shown that bacteria oxidize naphthalene through cis-1 (R), 2(S)-dihydroxy-1,2-dihydronaphthalene. The enzyme catalyzing this reaction is very unstable and the only reported attempts at purification achieved a two-fold increase in specific activity. Conditions for the preparation of active cell extracts and also for the preservation of enzymatic activity have now been established.

Cells of Pseudomonas NCIB 9816, after growth with naphthalene, were frozen for 24 hours and then broken by sonic disintegration. This treatment gave cell extracts with specific activities of 0.12-0.13 μmoles of oxygen-consumed/min/mg of protein. These values are four to five times higher than previously reported naphthalene dioxygenase activities from this organism. Fractionation of the crude cell extract with ammonium sulfate yielded two fractions, 30-50 percent and 50-75 percent, each inactive in the presence of naphthalene and NADH. However, combination of the two fractions resulted in the recovery of 81 percent of the activity present in the original crude extract. Naphthalene dioxygenase, unlike toluene dioxygenase, does not require the addition of ferrous ions for maximal activity and can utilize either NADH or NADPH as the electron donor.

A Beijerinckia species and three Pseudomonas species were isolated with p-chlorobiphenyl as the sole source of carbon. Growth occurred at the expense of the unsubstituted ring since all four organisms produced stoichiometric amounts of p-chlorobenzoic acid. A mutant strain of Beijerinckia (B836) oxidized p-chlorobiphenyl to cis-1,2-dihydroxy-3-(4-chlorophenyl)-3,5-cyclohexadiene. This compound was isolated in crystalline form and identified. Substitution of chlorine at the para-position in both aromatic rings (4,4'-dichlorobiphenyl) made the molecule recalcitrant to oxidation by all of the bacterial isolates.

Food Additives and Adulterants

Peroxides in Foods

Peroxide-initiated reactions have been investigated by Drs. M. Karel and P. M. Newberne (Massachusetts Institute of Technology) to determine the possible mechanism and health consequences of lipid peroxidation in foods.

Free radical formation in proteins, free amino acids, and in glutathione exposed to oxidizing lipid has been studied by means of electron spin resonance and by selected chemical methods. Free radicals were produced in model systems composed of oxidizing methyl linoleate or arachidonate and various proteins. These radicals are localized in the proteins. The ESR signals of lysozyme exposed to oxidizing lipids and γ-radiation were produced only after denaturation and reduction. Radical concentrations in crystalline lysozyme irradiated in air with one and two Mrad of γ-rays were comparable to those obtained after incubation with oxidizing linoleate.

In systems composed of lysozyme and oxidizing linoleate, production of radicals generally paralleled initial increases in lipid peroxide values, but reached and mantained highest levels after peroxide values fell to relatively low levels. In analogous systems composed of lysozyme, casein, or ovalbumin and t-butyl alkoxy and peroxy radicals, the alkoxy radicals were the most effective transfer species, initiating radical production in the proteins within a few hours and subsequently producing highest radical concentrations. Peroxy radicals and a 3:1 mixture of peroxy and alkoxy radicals were less efficient.

All the amino acids present in lysozyme were reacted in crystalline form with oxidizing methyl linoleate. Of the non-sulfur amino acids, only tryptophan, lysine, arginine, and histidine produced detectable ESR singals. The ESR spectra of tryptophan and arginine were similar to those obtained after irradiation in air. All the amino acids exhibited evidence of some spin density on nitrogen atoms, but only in arginine was there sufficient detail to propose specific radical sites.

Of the sulfur amino acids, cystine reacted with oxidized linoleate to a limited extent. ERS spectra indicated spin localization predominantly on a carbon atom. This apparent lack of reactivity arises from steric restrictions to attack at the S-S bond. Cysteine and glutathione readily produced ESR singals which were nearly identical and indicated the presence of both alkyl and sulfur radicals.

The following proteins were exposed to oxidized linoleate: lactalbumin, gliadin, bovine serum albumin, ovalbumin, thiolated gelatin, myoglobin, catalse, urease, and casein. Urease did not give detectable ERS signals. This anomalous behavior may be attributed to very rapid radical recombinations due to its abnormally high sulfhydryl content (82/molecule). All the other proteins exhibited strong radical signals. The g-values ranged from about 2.0030 to 2.0060, reflecting variations in spin density distributions along the protein backbones.

Although sulfur radicals were readily formed in the sulfhydryl-containing proteins ovalbumin, thiolated gelatin, and catalase, the disfulfide proteins BSA and gliadin showed evidence of sulfur radicals only after extended periods of incubation, while no sulfur resonance was detected in lysozyme and lactalbumin.

Interactions of Dietary Selenium

Dr. Newberne has continued his extensive studies on interactions among environmental agents (especially in foods). He has examined the effects of selenium on the LD_{50} of aflatoxin and found that too little or too much selenium enhances the toxicity of aflatoxin and that selenium requirement is significantly increased in the presence of dietary aflatoxin. Based on these results, he has initiated long-term studies to test effects in rats of various selenium dietary concentrations on chemical induction of tumors.

During the next year he plans to test the effects of dietary selenium upon other carcinogens including AF, since there is a considerable body of knowledge about its metabolism. In proposing an anticarcinogenic mechanism for selenium, it is hypothesized that selenium, as a part of glutathione peroxidase, may serve to destroy peroxides which can initiate highly reactive free radical or epoxide carcinogen metabolites. Epoxide metabolites have been implicated as the forms binding DNA, RNA and protein in aflatoxin and 3,4-benzyprene carcinogenesis. Thus the role of the glutiathione peroxidase system as an induceable antioxidant will be studied and compared to that of other antioxidants such as BHT and BHA on *in vitro* carcinogen metabolism.

Interest in lipotrope deficiency has two-fold significance since lipotrope deficiency has been demonstrated to enhance tumorigenesis and S-adenosyl methionine has been suggested a methyl donor in selenium metabolism. Di- and trimethyl selenides are less toxic to animals than sodium selenite and are the major excretory products. Tests will be made on effects of lipotrope deficiency upon selenium metabolism and toxicity.

Allyl Alcohol

During the past year, studies were initiated by Dr. Sheldon Murphy *et al.* (Harvard University) on the influence of carboxylesterase inhibitors on the toxicity and metabolism of esters of allyl alcohol. Several of these esters are used as food flavoring compounds. Allyl alcohol is known to be hepatotoxic, as is acrolein which is formed by oxidation of allyl alcohol. It would appear that at least two metabolic steps would be required for expression of allyl acetate toxicity; e.g., hydrolysis of the ester and subsequent oxidation. Preliminary investigations were conducted to examine first the importance of hydrolysis in modulating allyl acetate toxicity. The preliminary experiments indirectly suggest that carboxylesterases play a role in the initial metabolism of allyl acetate to a hepatotoxic agent.

Penitrem A

Etiology and pathogenesis. Dr. Benjamin Wilson (Vanderbilt University) has continued his studies on mycotoxins and phytotoxins. A preparative high speed liquid chromatograph was successfully employed in the purification of penitrem A, the principal neurotoxin from *Penicillium crustosum*. Several hundred milligrams of toxin were purified and given to Professor Palvo Stern in Yugoslavia, who is currently studying the mechanism of action of penitrem A in the central nervous system of mice.

The effect of i.p. administered penitrem A on dogs of both sexes and mixed breeding was determined by serum tests, by observation of clinical signs and survival times, and by evaluation of gross and microscopic lesions. Alkaline phosphatase, glutamic-oxaloacetic transaminase, lactic dehydrogenase and creatinine phosphokinase activities increased; survival time varied in relation to dose of mycotoxin administered. Consistent clinical features were anorexia, weight loss, emesis, tenesmus, rectal temperature to 108°F and dehydration. Tremors appearing as early as 10 minutes after dosing and ending in chronic or tetanic convulsions were observed in dogs receiving 0.5 mg/kg or more of penitrem A. Blood uric acid and cholesterol exceeded the normal range. Gross pathologic changes frequently observed were subserosal and submucosal hermorhages in some organs of the thoracic and peritoneal cavities. Dose-related liver damage varied from massive necrosis in dogs receiving 2.5 to 5.0 mg/kg penitrem A to no pathologic alterations in dogs given 0.125 mg/kg. Treatment with pentobarbital was successful for penitrem A poisoning if administered within one to two hours after exposure to the mycotoxin.

The effect of penitrem A on nucleic acid, protein, glycogen and lipid in brain and liver of mice was investigated. A single dose of penitrem A (2.5 mg/kg, ip) in propylene glycol caused in initial decrease in liver DNA of 53 percent. Total liver glycogen decreased by 68 percent by six hours after treatment but returned to pretreatment levels by the end of the 48 hour experiment. Total liver lipid increased 178 percent at 24 hours. A disappearance of free fatty acids concomitant with an increase in liver triacylglycerols was detected. Protein which was reduced 38 percent at six hours was the only consistuent measured in brain homogenates that was affected. In fasted animals, liver glycogen was elevated over a 72 hour period. Four consecutive daily ip administrations of penitrem A totaling 4.0 mg/kg, resulted in reduced food consumption and suppression of growth. Liver lipid and DNA were significantly lower whereas glycogen was significantly increased.

Previous studies have suggested that the toxin either inhibits glycine formation or action at inhibited interneurons, thus accounting for the sustained tremors and convulsions induced by the tremorgen. The effect of penitrem A injection on glycine levels in the brain and medulla spinalis of mice revealed a significant drop in glycine levels of the brain and spinal cord lasting for six or more hours. The effects of toxin pretreatment of animals on the reactions usually induced by several CNS drugs or stress conditions was also examined. The results obtained in several cases (but not all) were consistent with the hypothesis that glycine levels were reduced by penitrem A.

Daily administration of neurotoxin was found to prolong the lives of mice given tumor transplants of two different types of cancer cell lines. Although the survival spans were prolonged, the size of the tumor masses was not significantly different in toxin treated animals as compared to cancer controls. Since there is evidence that glycine is a key metabolite in tumor development, these observations were accepted as suggestive evidence that the toxin could act as an inhibitor of glycine formation.

Ipomeanol

Mechanism of action. Dr. Benjamin Wilson has carried out additional studies on the mechanism of action for the lung toxins of stressed sweet potatoes. In vitro studies on covalent binding of radioactive 4-ipomeanol showed that subcellular structures of rat lung tissue in the presence of NADPH generating system bind more radioactivity than those of the liver. Microsomes bound more than other cellular fractions.

3-Methycholanthrene (3MC) pretreatment of rats was responsible for shifting the site of toxin binding somewhat from the lung to the liver with the result that lung damage was diminished, and the liver exhibited hepatocellular necrosis. Kinetic studies on the covalent binding to lung and liver microsomes showed that 3MC pretreatment increased the V_{max} in the liver microsomes, although the K_m was unchanged. This suggests that 3MC can increase the rate of formation of reactive metabolite in the liver with a resulting increase in toxicity for that organ. The in vitro covalent binding to lung enzymes was unchanged, although the in vivo binding to lung was markedly diminished.

Mechanism of tolerance and protection against ipomeanol. Tolerance development to 4-ipomeanol challenge, as the result of several daily injections of sub-lethal doses, was shown to correlate with decreased binding of activated toxin to the lungs. Both in vivo and in vitro microsomal binding of toxin was decreased in the lungs and livers of pretreated animals. Kinetic studies showed that the K_m was unchanged, whereas the V_{max} was markedly decreased in microsomes of both livers and lungs of 4-ipomeanol pretreated animals. Toxin pretreatment also decreased the metabolism of benzphetamine and aniline by rat lung and liver microsomes. These results indicated that 4-ipomeanol pretreatment decreased mixed function oxidase activity which is apparently required for toxicity.

It was shown in a series of experiments that glutathione markedly inhibits covalent binding of 4-ipomeanol to washed microsomes. Diethylmaleate pretreatment in vivo made rats more susceptible to toxin. Also, this phenomenon was correlated with increased covalent binding of toxin to lung and liver protein in vivo. However, the lung remained the prime organ of toxic attack.

Structure-activity relations. Previous work has established that phenyl and methyl analogs of 4-ipomeanol are not toxic when injected at levels of 800 mg/kg in mice. This indicated the importance of the furan moiety for toxicity. Additional experiments have shown that these analogs do not covalently bind to any significant extent to lung or liver microsomes in vitro through an NADPH-dependent mechanism. Also, pretreatment of rats with the analogs did not induce tolerance to 4-ipomeanol challenge. Thus, the furan moiety is essential for metabolism, toxicity and tolerance induction by 4-ipomeanol.

Rubratoxin B

Etiology and pathogenesis. Dr. Wallace Hayes (University of Mississippi Medical Center) has made advances in several aspects of mycotoxin research under a

rather broad program. Bioproduction, purification, and preliminary structural elucidation of rubratoxin B, a hepatotoxic metabolite produced in food materials and in semisynthetic media by Penicillium rubrum, have been accomplished. Studies concerning the molecular mechanism of action of this mycotoxin in the mouse and the rat have shown that both glycolysis and protein biosynthesis in liver tissue are disrupted by extremely low doses.

Approximately 140 microorganisms, including bacteria, algae, fungi and protozoa were screened against rubratoxin B. Bacillus subtilis, Tetrahymena pyriformis, and Volvox aureus were three of the organisms found to be sensitive to this mycotoxin. Studies with B. subtilis have shown that protein and RNA content of growing cells are altered by rubratoxin B but DNA is apparently not affected.

Alterations in liver polysome profiles, in specific RNA levels, and in RNAase activity after administration of rubratoxin B have been determined. In addition, spectral studies on binding of rubratoxin B to nucleic acids have also been undertaken. A decrease in the polysome peak and an increase in the monomer and dimer peak were observed in animals treated with rubratoxin B and sacrificed at 0.5 hours after dosing. A maximum increase of monomer plus dimer peak of 45 percent was observed by one hour. Reaggregation as indicated by progressive increases in the area of the polysome profile and the return of monomer plus dimer areas to control values had begun by three hours post dosing. By 48 hours profiles of treated and control mice were essentially identical.

The in vitro effect of rubratoxin B on the electron transport system of mouse liver mitochrondria was investigated. Rubratoxin B depressed oxygen consumption 73 percent at a concentration of 11.3×10^{-4}M. A 50 percent inhibition was observed with concentrations as low as 0.83×10^{-4}M. At a concentration of 2.5×10^{-4}M, rubratoxin B depressed oxygen consumption a minimum of 40 percent in ADP-coupled mitochrondria and 60 percent in DNP-uncoupled mitochrondria using either pyridine nucleotide linked substrates or succinate. Rubratoxin B appeared to invoke an inhibitory pattern similar to sodium azide and potassium cynide, both known inhibitors of electron flow at the level of cytochrome C_1 or C and the termination of electron flow. It appears that the principal site of action of rubratoxin B is on the electron transport system between cytochrome C_1 or C and the termination of electron flow.

Metabolism of rubratoxin. Work with radioactive rubratoxin B indicates the toxin was decarboxylated before being excreted in the urine. In addition the increased activity of β-glucuronidase indicates the possibility of conjugation. It is postulated that rubratoxin B is metabolized to a less toxic metabolite followed by decarboxylation, with the remainder of the molecule conjugated prior to excretion. Studies currently underway on castrated animals indicate that the LD_{50} in these animals is decreased, which would support this hypothesis.

Studies were undertaken to determine if specific enzymes are involved in alteration of rubratoxin B. The study suggests that rubratoxin B is

metabolized in the endoplasmic reticulum because of its adverse effect on NADPH oxidase activity. The fact that the activity of p-nitroanisole demethylation was inhibited leads to the conclusion that at the longest time postdosing, the pathways for the metabolism of rubratoxin becomes overloaded. Whether there is subsequent conjugation of rubratoxin before excretion or decarboxylation was not ascertained. However, earlier research indicates that the major excretion product was carbon dioxide. Rubratoxin B did not affect any of the components of the microsomal electron transport chain.

Ochratoxin A (OA)

Interaction with proteins, etc. The in vitro interaction of OA with different proteins or enzymes was studied by Dr. Fun Chu (University of Wisconsin) by an equilibrium dialysis method using ^{3}H-OA as the testing reagent. The results confirmed a previous investigation that OA interacted with arginase and serum albumin. In addition, strong binding between OA and thrombin, catalase, and weak binding between OA and deoxyribonuclease, leucine amino peptidase were observed. The significance of such interaction is presently under investigation.

A comparative study of the interaction of (OA) with bovine serum albumin has been completed. The interaction of OA with BSA was further confirmed by a circular dichroism study. OA generated a negative extrinsic Cotton effect for BSA at 390 nm. The effect reached a maximum when OA:BSA molar ratio in the solution reached 2.0, indicating that two moles of OA interacted with one mole of BSA. The extrinsic Cotton effect was abolished when 8 M of urea was present in the reaction mixture.

The optimal conditions for tritiation of ochratoxins were studied ^{3}H-ochratoxins A, B, and C were obtained after tritiation of the toxins with tritiated water in acetic acid and platinum at 80^{o} overnight. The toxins were purified by solvent partition and tlc with the recovery yield and specific activity being 80 percent and 2.6 - 3.0 Ci/mM, respectively.

Aflatoxins (AF)

Production. Approximately 121 fungus isolates, representing 29 species, were examined for aflatoxin production in conjunction with investigators at Vanderbilt University, Virginia Polytehcnic Institute, University of Mississippi, and the University of Georgia. This study indicated that only Aspergillus flavus and A. parasiticus are apparently capable of synthesizing aflatoxin. However, Dr. Barbara Joyce (Colorado State University) has demonstrated that AF may be formed in certain animals infected with AF-producing molds and the yeast Torula pintolopesii. Holtzman rats were inoculated with spores of A. parasiticus and different forms of AF were found in the urine, feces and various tissues of one of the rats. The common forms of AF were found in the urine of several Sprague-Dawley rats inoculated with T. pintolopesii isolated from several rats which had developed a terminal disease after inoculations of A. parasiticus. Three derivatives prepared from suspected B_1 gave R_f values identical to those of derivatives prepared from authentic B_1. Also, suspected B_1 and authentic B_1

chromatographed in four different solvent systems had the same R_f values in all systems. The same tests indicated that AF G_1 also was isolated from the Holtzman rat.

Pathogenesis of AF. Reproductive abnormalities were observed during the second and third months of the experiment in which rats were inoculated with the yeast at weekly intervals for one month. Some members of litters were stillborn, while several others died within a few days. Extractions of livers from two different litters showed small amounts of fluorescent material having an R_f similar to that of AF M.

Immune antisera against AF B_1 or kojic acid were prepared by injecting rabbits with the respective toxin-bovine serum albumin conjugate suspended in Freund's complete adjuvant for the first three injections and Freund's incomplete adjuvant for the remaining injections. The immunization schedule consisted of biweekly injections of 5 mg of the conjugate for six months.

An enzyme-linked immunosorbent analysis, using alkaline phosphatase as the test enzyme, has been developed. The concentration of rabbit serum albumin used in the diluent to obtain maximal sensitivity was 0.5 percent. As developed thus far, this test can detect 10^{-3} ng of AF B_1/ml. A similar enzyme-linked immunoabsorbent analysis has been developed to detect 5×10^{-2} ng of kojic acid/ml.

Transfer RNA methylase activity and capacity and histopathological changes were monitored by Dr. Gerald Wogan et al. (Massachusetts Institute of Technology) during the course of aflatoxin B_1-induced hepatocarcinogenesis in the rat. In contrast to literature reports, no significant increase in tRNA methylase activity was noted throughout the experiment. However, two distinct phases of increased tRNA methylase capacity were noted during the weeks 6 to 9 and 24 to 29. Increase in the aflatoxin dose resulted in acute toxic liver damage and death of the animals after three weeks of dosing. However, in this case, marked elevations in both the tRNA methylase activity and capacity occurred at least as early as one week after dosing. Individual liver lesions from animals continuously fed a 2 ppm diet were excised and tRNA methylase activities and capacities determined. Elevated methylase function was also noted in hyperplastic pre-neoplastic nodules as well as in non-nodular surrounding liver. An additional set of experiments was performed relating methylase activity and capacity to the effects of a single acute dose of aflatoxin. Elevated dose-independent methylase activity (approximately 40 percent) was noted at least as early as three days after dosing and gradually declined towards control levels at the end of the experiment (21 days). Methylase capacity was measured as a function of aflatoxin dose seven days after i.p. injection. A linear dose-dependent relationship with values elevated as high as 100 percent over the control levels was noted.

Biogenesis of AF and other mycotoxins. Dr. Dennis Hsieh (University of California) has continued studies on the pathways of biosynthesis to toxic fungal polyketides, their mechanisms of action, and control of mycotoxins in

foods. In studies on incorporation of radioactivity of ^{14}C-labeled averufin (AR), norsolorinic acid (NA), and acetoacetate into sterigmatosystin (ST) or aflatoxin (AF), it was found that averufin was readily converted into ST and AF by the appropriate cultures, whereas NA, though structurally related to AR, is a common intermediate in the biosynthesis of ST and AF, and the AF-biosynthetic pathway is probably operative up to the formation of ST.

^{14}C-Acetoacetate was incorporated into AF with an efficiency comparable to acetate. Addition of AR into the culture increased the yield of ST but did not increase the incorporation of acetoacetate. The C_4-compound acetoacetate, therefore, did not seem to replace the C_6-side chain of AR and to be selectively incorporated into the bisfuran ring system of AF. The experimental evidence does not indicate AF to be synthesized by fusion of preformed C_4 and C_{14} units, but rather, indicates the involvement of a single polyketide intermediate in the biosynthesis.

Analysis of labeled AF confirmed the polyketide orgin of AF and indicated that the bisfuran ring system in the molecule is derived from the C_6-side chain of AR. The experiment indicates that AF is synthesized through a C_{20} polyketide AR rather than by fusion of a C_4 and a C_{14} performed polyketide unit.

AF-producing cultures of A. parasiticus, when treated with dichlorvos, excreted an orange pigment (OP) which could be converted into AF by the untreated mycelia. The OP was partially identified as an acetyl derivative of versiconol type compound. In the presence of dichlorvos, ST was converted into AF, but AR was converted into OP instead of AF. Therefore, dichlorvos appears to block an enzymatic step in the AF-biosynthetic pathway which lies beyond AR but before ST. Total identification of OP is in progress.

The present study has considerably advanced understanding of the biosynthesis of sterigmatocystin, aflatoxins, and related mycotoxins. It reveals that toxic fungal polyketides of diversified structures and biological activity, in fact, can be considered as a group on the basis of their biogenetic relationship. The related biogenetic origin of these mycotoxins suggests a possibility of collectively controlling the formation of a spectrum of mycotoxins with chemical agents such as dichlorvos.

Interactions and binding of AF. Several grantees have conducted extensive studies to elucidate interaction mechanisms and sites of AF, with the aim of clarifying its toxicity and carcinogenic mechanisms.

Dr. Fun Chu (University of Wisconsin) has demonstrated that aflatoxin B_1 can form charge-transfer complexes with electron-donating organic compounds, including amino acids. In order to find out whether this charge-transfer interaction plays any role in the toxicity of aflatoxins, the interaction of four different aflatoxins with tryptophan was tested. All four aflatoxins reacted with tryptophan to some extent but B_2, G_1, and G_2 to a lesser degree than B_1. The equilibrium constants for tryptophan-aflatoxins (1:1 complexes) B_1, B_2, G_1, and G_2 were found to be 22, 16, 10, and 5 liter per mole, respectively.

Interaction of aflatoxin B_{2a}, the hemiacetal of aflatoxin B_1, with amino acids and proteins has been demonstrated by spectrophotometric analysis, spectrophotometric titration, equilibrium dialysis, separation of the interaction complex from free reactants by tlc at pH 7.0, and by the isolation of stable conjugates upon reduction with sodium borohydride. At pH 7.8, the spectrum of B_{2a} shifted to a longer wavelength (from 370 nm to 400 nm) when either glycine or ovalbumin was present, but did not shift in the presence of N-acetylglycine. Likewise, both glycine and ovalbumin altered the dissociation constant (pK) of the phenolic group of B_{2a} but N-acetylglycine did not. The empirical formula for the complex was B_{2a} $(Phe)_2$ when ^{14}C-phenylalanine (Phe) was used in the interaction. An equilibrium constant for the B_{2a} $(Phe)_2$ system was found to be 1.82×10^7 liter M^{-1} at pH 7.0. The complex was stable in neutral and slightly alkaline pH but dissociated in acid in contrast to the reduced adduct, which was relatively stable under acidic conditions. The interaction was also pH-dependent, with a higher degree of interaction as basicity increased. The results indicate that the α- and ε-amino groups of amino acids and proteins and the aldehyde groups of the phenolate ion form of B_{2a} were essential for the reaction, and support the proposed Schiff base formation mechanism for the interaction.

An aflatoxin B_{2a}-protein adduct which was produced by reduction of reaction mixture with $NaBH_4$ is currently using for the production of antibody against aflatoxin. The presence of aldehyde functional groups in aflatoxin B_{2a} at neutral and alkaline pH was confirmed by the formation of reduced aflatoxin B_{2a} with $NaBH_4$.

Reduced aflatoxins B_1 and B_2 were prepared with $NaBH_4$. The new derivatives have different chromatographic properties, showed fluroescent under uv, and were identified as the trihydroxy derivatives of aflatoxins B_1 and B_2. Since the reaction was almost quantitative, formation of these derivatives has been recommended as a confirmatory method for aflatoxin analysis.

With the very recent availability of high specific activity ^3H-aflatoxin, Dr. Wogan et al. have investigated the interactions of aflatoxin with rat liver macromolecules. Incubation of ^3H-aflatoxin with liver cytosol for two hours at 0^oC, followed by a 30 minute "activation" at 20^o in the presence of 0.15 M NaCl, has resulted in the formation of aflatoxin-macromolecule complexes separable by Sephadex G-25 column chromatography of the cytosol. The addition of a metabolism step by the incubation of the ^3H-aflatoxin with a 10,000 x g liver supernatant in the presence of NADPH generating cofactor for 15 minutes at 37^o promotes increased binding of the aflatoxin and quantitative differences in the eluted peaks, but no alteration in the elution patterns.

As a monitor of the biological activity of these complexes, each of the peaks (I-macromolecular:M. W. >5000; II and III:M. W. between 1000-5000) has been incubated for two hours at 0^o with liver nuclei. Peak I showed some specific binding capacity (about two orders of magnitude lower than that of steroid receptors) with a Scatchard analysis of the data yielding a concave plot possibly suggesting three classes of receptor sites: a small number of

high affinity sites, some intermediate affinity sites, and a large number of non-specific, low affinity binders. Peak II showed no binding capacity and Peak III showed a fairly high degree of non-specific (non-saturable) binding ability as evidenced by a horizontal Scatchard plot.

Preliminary investigations have been designed to determine the nuclear distribution of ^{3}H-aflatoxin B_1 administered in vivo to male Fischer rats. Weanling rats were dosed i.p. with 250 μC ^{3}H-aflatoxin B_1 diluted with carrier aflatoxin (final dose: 1 mg/kg). The animals were sacrificed after two hours and purified nuclei prepared. Approximately 3-5 percent of the total liver homogenate radioactivity was present in the purified nuclear pellet. The nuclear fractions were further sub-divided for counting into nucleoplasmic material, dissociated chromatin protein, DNA and residual DNA-bound protein, corresponding to 14.0 percent, 10.2 percent, 45.5 percent and 2.7 percent of the total nuclear radioactivity, respectively. The nucleoplasmic and chromatin protein fractions were exhaustively dialyzed against water. Typically, 50 percent of the nucleoplasmic fraction was dialyzable; whereas, only 10-15 percent of the chromatin protein was dissociable.

The original hypothesis was that the cellular interactions of aflatoxin B_1 might be reflected in alterations of the normal mechanism of glucocorticoid action at the level of glucocorticoid binding to cytosol receptors, the temperature-dependent transformation of the steroid-cytosol receptor complex, or the nuclear binding of the transformed steroid receptor complex.

The first requirement was to demonstrate the existence of specific, high affinity, single-class binding of ^{3}H-dexamethasone to cytosol receptor proteins by Scatchard analysis. This was successfully confirmed. Similar single-class high affinity binding of the ^{3}H-dexamethasone-cytosol receptor complex to nuclei was also successfully accomplished with saline, DMSO and aflatoxin B_1-treated rat liver preparations. Aflatoxin-treated rats have an apparent concentration of nuclear binding sites approximately 30 percent lower than that found in saline or DMSO treated rats. Because the aflatoxin effect is consistent regardless of whether aflatoxin pre-treated rat liver cytosol receptor or nuclei are used in the assay, the suggestion is that aflatoxin does not exert a direct effect on the formation of the glucocorticoid-cytosol receptor complex or on its ability to be transformed to an "active" configuration, but rather an aflatoxin substituent, present in the cytosol, has the ability to interact with DNA-chromatin at sites common to at least some of those occupied by glucocorticoids.

Metabolism of AF. Dr. Dennis Hsieh (University of California, Davis) has studied the metabolism of aflatoxin B_1 in the Rhesus monkey in an effort to find mechanisms characteristic of human metabolism. Incubation of AFB_1 with monkey liver microsomal preparations in the presence of an NADPH regenerating system resulted in the formation of two main metabolites. One was AFM_1 (1-3 percent of the substrate) and the other was a novel metabolite (16-52 percent of the substrate) which was isolated and identified as an isomer of AFM_1, with the hydroxyl on the carbon atom β to the carbonyl of the cyclopentenone ring. The name aflatoxin Q_1 (AFQ_1) was proposed for this metabolite

In comparisons of metabolism by human and Rhesus monkey liver tissue obtained by biopsy, metabolism of AFB_1 by the homogenates of these liver specimens showed great similarity in that they all transform AFB_1 to AFQ_1, M_1, B_{2a}, aflatoxicol, and aflatoxicol-H_1, with AFQ_1 being the predominant metabolite. Aflatoxicol-H_1 is a newly identified metabolite characterized as hydroxylated aflatoxicol with the hydroxyl at the carbon β to the alcohol group of the cyclopetenol ring (or reduced AFQ_1).

Dr. David Yourtee (Cancer Research Center, Columbia, Missouri) has studied the metabolism of aflatoxin by human liver in vitro. A variable-multiple pattern has been revealed, involving a total of 11 metabolites. Six human metabolites common to the lower species have been substantiated. Three heretofore unclassified derivatives occurred and two additional unknowns have been isolated induced by additive effect. Individuals range from three to six metabolites with a pattern not necessarily dependent on disease state (maximum and minimum number of metabolites was produced by metastasized liver). Both oxidized products (AFQ_1, AFM) and reduction products (aflatoxicol, AFB_2) have been encountered. Aflatoxin M is a product of minor to intermediate relative quantitative importance. Aflatoxin Q_1 has been the more frequently observed. Aflatoxicol and its oxidized derivative $AFLH_1$ have appeared frequently and are of quantitative importance but have not been observed together. Although no aflatoxin metabolite should be considered a priori as toxicologically unimportant in the human, other metabolites have generally appeared sporadically in relatively lower amounts.

Other studies show that co-substrates can lead to a change in pattern of AFB_1 metabolites and rate of production by human liver microsomes. Pattern of shifts appears selective for the additive. Methionine, a protective agent in experimental animals, induces a shift in pattern, inducing unknown metabolites of less polarity than AFB_1. Suppressed production rate and number of AFB_1 metabolites were found when NADP was withheld, suggesting that mixed function oxidases are involved in human liver conversion of the parent toxin. AFB_1 turnover is substantially reduced by nicotinamide, a known mixed function oxidase inhibitor. Nicotinamide did not induce metabolites promoted by methionine in the same biopsy but did induce three metabolites of different polarity. For samples studied, known oxidative and reduction detoxification products AFB_2 and AFO_1 were not produced in the presence of nicotinamide.

Miscellaneous Mycotoxin Studies

Some mycotoxins have been evaluated with respect to effects on the reticuloendothelial function of uptake and degradation of a soluble, denatured protein in phagolysosomes isolated from mouse liver and kidneys. Treatment with citrinin, penitrem A, sterigmatocystin and zearalenone produced significant inhibition of protolysis in kidney but not in liver phagolysosomes. Moniliformin and T-2 toxin had no detectable effect on lysosome formation or function in either tissue. Of the agents which affected proteolysis in the liver, none caused a significant increase in liver phagolysosomal fragility as measured by osmotically releasable radioactivity or inhibited endocytosis of labeled albumin into liver cells. The phagolysosomal toxicity appeared to be more toward kidney than liver. Both sterigmatocystin and zearalenone

caused an increase in kidney phagolysosomal fragility at early times after treatment and all agents except penitrem A caused a decrease in uptake of labeled albumin into kidney cells. Citrinin labelized kidney phagolysomes in vitro but not in vivo; therefore, citrinin appears to be converted in vivo to a non- labelizing form which may be the agent responsible for its antilysosomal activity. Penitrem A inhibited proteolysis in both liver and kidney but did not alter uptake of labeled protein nor increase ability of the particles. Further, penitrem A had little effect on cathepsin activity.

Mutagens and Carcinogens

Nitroso Compounds

Because of the mutagenicity and carcinogenicity of N-nitroso compounds as a class and the possible formation of such compounds from nitrogen oxides, many projects dealing with these compounds are being supported. Individuals may be exposed to nitroso compounds through a variety of possible sources, which include: (1) laboratory and pilot plant exposures to dimethylnitrosamine; (2) production of unsymmetrical dimethylhydrazine; (3) the use of nitroso compounds as rubber additives; (4) commerical production of nitrosamines; (5) research scientists using nitroso compounds; (6) ingestion of foods containing nitroso compounds; (7) formation of nitrosamines through the interaction of durgs with nitrites; and (8) nitrosamines formed by ingestion of nitrates in water systems.

Reactions of nitrosamines. Drs. S. R. Tannenbaum and M. C. Archer (Massachusetts Institute of Technology) have conducted extensive studies on the chemistry and biological activity of nitrosation reaction products. Diethylnitrosamine and nitrosopiperidine incubated separately for five hours in the Udenfriend system in sealed flasks with oxygen in the headspace yielded two major peaks in addition to the original diethylnitrosamine. The two peaks were shown by spectroscopy and synthesis of authentic material to be due to the syn and anti conformers of N-nitroso-2-(ethylamino)-ethanol. Nitrosopiperidine yeilded only one major peak in addition to the original nitrosamine. The peak was identified as nitroso-4-piperidone by spectral analysis and synthesis of authentic material.

The products formed by reaction of nitrosamines in the Udenfriend system are similar to those found with simple hydrocarbons and probably arise by insertion of a highly reactive exenoid species directly into carbon-hydrogen bonds. Further oxidation of the alcohol so formed would yield the ketone. It should be noted that in no case were any reaction products isolated when nitrogen was used in the headspace gas.

Mutagenicity of nitrosamines. These investigators have utilized the tester strains of S. typhimurium together with induced rat liver preparation to investigate the mutagenic activity of a series of nitrosamines. It was found that the nutritional status of the rat prior to sacrifice is an extremely important parameter in the induction of enzymes which activate nitrosamines. Fed animals activated nitrosamines to produce mutagenic species. The fasted induced animals had activity only with DEN. In all cases when the 9000 x g

supernatant was omitted or heat-inactivated, no mutant colonies were formed. It thus appears that the system using S. typhimurium histidine requiring mutants together with microsomes from the livers of phenobarbital-induced rats which have not been starved will provide a useful system to study the activation of nitrosamines and the system may provide a bioassay for carcinogenic members of the series.

Spermidine tetrahydrochloride was twice recrystallized from ethanol-water and nitrosated the residue was extracted into acetone. This crude, multicomponent reaction mixture was then assayed for mutagenicity using the S. typhimurium system. This reaction mixture was mutagenic for all three tester strains of S. typhimurium without activation by rat liver microsomes. In all three strains, however, the size of the ring of revertants was qualitatively greater in the presence of the microsomal system. Thus there appears to be chemical species produced by nitrosation of spermidine which are mutagens without requiring prior metabolic activation which are stable enough to survive chemical work-up of the reaction mixture. There also appear to be other chemical species in the mixture which become mutagenic after metabolic activation, thus enlarging the zone of mutant colonies. Since all three strains were mutated, the reaction mixture must contain a variety of species capable of interacting with DNA in different ways. Attempts are being made to identify the specific active components of the mixture.

Drs. Gomez and Sinskey (Massachusetts Institute of Technology) have investigated damage to DNA by nitrosamines in single-cell bacterial and mammalian systems. They have established that N-nitrosomorpholine and N-nitropyrrolidine are metabolized by rat liver homogenates to mutagens active on Salmonella typhimurium. The resulting mutations seem to have been caused by base-pair substitutions and, therefore, are believed to be due to alkylations. A variety of pesticides were also tested and found to be negative. Among those tested were chlordane, DDT, parathion, linuron, orthene, and karathane. These compounds were negative with and without the rat liver microsomal activation and also in the repair test.

Chang human liver cells treated with N-methyl-N'-nitrosoguanidine (MNNG) and N-nitrosomorpholine (NM) exhibited DNA breaks and repair synthesis was detected. In addition, cells were exposed to the folic acid analog, 2-hydroxy-N^{10}-nitrosofolic acid, which did not cause damage to the DNA. Arlacel A, a co-carcinogen, prevented repair synthesis in cells treated with NM and MNNG. Additional studies have indicated that NM treatment of Chang liver cells leads to a heavy sedimentating DNA component. Experiments are proposed to determine the nature of this complex.

Diacetylfuroxan (DAF), a product of the reaction of nitrogen tetroxide with acetone, was tested against Salmonella typhimurium and mammalian cells in culture. DAF inhibited growth and inactivated an uvrB mutant of S. typhimurium to a greater extent than the wild type uvr+. In addition, DAF sensitized cells of S. typhimurium to γ-radiation. Although DAF did not exhibit any mutagenic activity against the strains tested, the fact that it is more active against an uvrB mutant that uvr+ indicates it may interact with cellular DNA.

Herbicides

Chromosomal breakage and loss. Dr. Mary Murnik (Western Illinois University) is testing the mutagenic activity of the more commonly used herbicides, using a Drosophila test system. Assays are for chromosomal breakage and loss and for sex-linked recessive lethals. The herbicides being tested are terbacil simazine, bromacil, alachlor, atrazine, butylate, cycanazine, linuron, propachlor, trifluralin, and vernolate. It was found that larval ingestion of 0.3 percent bromacil or 0.2 percent terbacil by weight results in a significant increase in unhatched eggs. Chromosomal loss and deletion assays indicated that there was an increase in dominant lethal mutations and that these were due to chromosomal nondisjunction and loss, not to breakage. The preliminary results obtained so far do not indicate that these herbicides induce recessive lethal mutations. Tests on the other herbicides mentioned are not yet complete.

Aflatoxins

Effects on non-histone nuclear proteins. Drs. G. N. Wogan and W. F. Bushy have conducted extensive studies dealing with various aspects of carcinogenesis. In studies on neoplastic alterations in non-histone nuclear proteins they have demonstrated that a major nuclear protein (NHP-01) is either entirely absent or very significantly decreased in aflatoxin (AFB_1)-induced rat liver tumors. Additionally, it appears that several nuclear proteins of the tumor cells are less strongly bound to the DNA (or DNA-histone complex) since they can be extracted with lower ionic strength buffers than the corresponding components in the normal tissue. This preliminary finding is of particular importance since recent work by Gronow and Thackrah (1974) indicates that a non-histone nuclear protein (M. W. 17,500) is absent or significantly decreased in diethylnitrosamine (DEN) induced rat liver tumors. At present, these two proteins appear to be non-identical. In view of the potential importance of the NHP-01 nuclear protein in chemical carcinogenesis, they have planned to put more emphasis on its study and function at the initial stage of the proposed work.

A novel approach to the study of carcinogen interaction with cellular macromolecules arose from earlier observations that aflatoxin B_1 inhibits the hydrocortisone-stimulated induction of tyrosine aminotransferase and tryptophan pyrrolase in rat liver in a manner similar to actinomycin D.

Miscellaneous Mutagens

Dr. Frederick Sobels et al. (University of Leiden, Netherlands) has initiated a rather extensive program for testing the induction of genetic damage by chemical mutagens. Twelve potential mutagens [AF-2; H-193; vinyl chloride; NO-carbaryl; NO-benzthiazurone; methyl-carbamoyl-imidazole; 2-chloroethyl-carbamoyl-imidazole; CNU (2-chloroethyl)-1-nitrosourea; CNU-ethanol; bis-CNU-ethane; bis-CNU-diphenyl-methane; and ethylene-glycol] and six reference compounds [azoxymethane; 2,4,6-triF-PDMT (triazene); 4-F-PDMT (triazene); 5-(3,3-dimethyl-1-triazene)-imidazole-4-carboxamide); NSC 45-388; natulan; 1,2-dimethylhydrazine] have been tested.

Among the chemicals so far tested, NO-carbaryl, 2-chloroethyl-carbamoyl-imidazole, CNU, and CNU-ethanol, are clearly active as mutagens with regard to recessive lethal induction in post-meiotic male germ cells of Drosophila after adult feeding of 1-5 mM for two to three days. CNU-ethanol is also mutagenic for the induction of dominant lethals and chromosome losses. Pilot experiments on AF-2, vinyl chloride, NO-benzthiazuron, and methyl-carbamoyl-imidazole, did not indicate any mutagenic response, but AF-2 showed poisoning effects on treated cells by blocking spermatogenesis.

Of the reference mutagen group, azoxymethane, NSC 45-388, natulan, and 1,2-dimethylhydrazine, were found to be highly active for recessive lethal induction in both meiotic and post-meiotic male germ cells. It is notweorthy that these agents require enzymic activation by microsomal enzyme systems, which are apparently present in Drosophila. Natulan has also been tested for the induction of dominant lethals and autosomal translocations, but did not show any activity. Attempts are now being made to perform a more detailed analysis of this mutagen specificity (dose-effect relationship for recessive lethals, chromosome total and partial loss).

An adult feeding technique was used to investigate the mutational spectrum of MMS, 2,4,6-triCl-PDMT, and DEN. The effects on mature spermatozoa were studied. The four compounds selected differ markedly in their mode of mutagenic action and chemical properties (mono- or polyfunctional; direct or indirect). With these agents, dose-effect studies were carried out, applying a wide concentration range of lowest effective concentration to the LD_{50}. With MMS and TEB, recessive lethals occurred at concentrations about one order of magnitude lower than that needed to induce significant numbers of dominant lethals and chromosome aberrations. 2,4,6-triCl-PDMT and DEN failed to induce breaks but were capable of giving high frequencies of recessive lethals--equivalent to one mutation per germ cell generation. These new findings show the high resolving power of recessive lethal tests as compared to the dominant lethals, translocations, and chromosome losses.

The lowest effective mutagen concentration of TEB could not be lowered by extending exposure from 24 hours to seven days. This experiment points to the existence of a "biochemical" threshold under the conditions used.

With a combination of four mutagens, 5×10^{-2} mM DEN, 2.0×10^{-2} mM MMS, 1.0×10^{-5} mM TEB, and 1.0×10^{-3} mM 2,4,6-triCl-PDMT, being ineffective when fed alone, no indication of an additive or synergistic interaction was observed. After doubling each mutagen concentration, mutation frequency was highest in the combination run (2.3 percent), also significantly increased for TEB (1.2 percent), but did not differ from control (0.2 percent) for MMS, DEN, and 2,4,6-triCl-PDMT.

Studies on Synthetic DNA Helices

Dr. Tso et al. have been able to synthesize a RNA helix of six base pairs (half of the helical turn). The helix has been analyzed thoroughly by proton magnetic resonance. The helix, with the sequence of AAGCUU, is formed at low temperature (10-20°) and high salt concentration (0.1-1.0 M NaCl). The

geometrical structure and the lifetime of the helix has been carefully determined by proton magnetic resonance studies. The interaction of caffeine with the helix in solution has been investigated by nmr and the results clearly indicated that the caffeine molecules intercalate between the two adenine base pairs at the end of the helix. The geometry of the caffeine in the intercalation can also be clearly delineated. This result implies such an interaction of caffeine with short RNA helices could take place in solution such as with short helices in the arms of tRNA and messenger RNA. Currently, Dr. Tso is in the process of finishing the synthesis of the DNA short helix with the sequence CCAAGC-TTGG.

These workers have developed an in vitro morphological transformation system using Syrian hamster embryonic fibroblasts. The system does not require a feeder layer and is suitable for mechanistic studies, particularly those concerning the metabolic pathway in which a precarcinogen is converted into the ultimate carcinogen.

They have also established a system in which embryonic fibroblasts can be assayed simultaneously for neoplastic transformation and somatic mutation as induced by the same chemical with the same dose. Early results indicate that neoplastic transformation is 10^2 to 10^3 more frequent than somatic mutation.

The significance of this lies in the fact that for the first time the direct relationship between somatic mutation and neoplastic transformation can be investigated and the findings so far do not appear to be consistent with the widely-held view that somatic mutation and neoplastic transformation are parallel or that transformation depends upon a mutation.

Studies on DNA Repair

Dr. John Little et al. (Harvard University) have examined the possibility that cell aging may be related to a decline in capacity to repair damaged DNA. The rate of rejoining of radiation-induced single strand breaks following exposure to 10^4 rads was studied by analysis of DNA sedimentation patterns from alkaline sucrose gradients. Normal diploid fibroblasts were grown from skin biopsies from a fetus and a young adult male. Growth rate, labeling index, and DNA repair capacity were determined every eight cell population doublings. Although more rapid at the very early passages, the rate of strand rejoining remained essentially constant throughout most of the in vitro lifespan of the cells. As the cultures became senescent, however, the post-irradiation sedimentation patterns became multimodal, suggesting the appearance of cells with reduced repair capacity.

DNA repair was studied also in skin fibroblasts from four patients with clinical symptoms of the Hutchinson-Gilford progeria syndrome of precocious aging. In two of these cases the rate of strand rejoining was similar to that of normal cells. In the other two it was markedly reduced; the amount of repair found in normal cells by 15-20 minutes post-irradiation did not occur until 60 and 120 minutes respectively in these progeric fibroblasts. When these cells were transformed by SV-40 virus, however normal repair capacity

returned. Separate experiments indicated that the rate of DNA strand rejoining was not simply related to the rate of cell proliferation.

Studies on Inverted Tandem Duplication of Chromosomes

Dr. George Yerganian (Sidney Farber Cancer Center) has made considerable progress in studies of mutagenesis and carcinogenic potentiation utilizing cells from the Chinese and the Armenian hamster. It was found that reticular cell neoplasms feature a rare and unique intrachromosomal rearrangement, namely, inverted tandem duplication. The mechanism leading to inverted tandem duplications has been recorded in detail, and is subject to genetic control. The specific sites are also predisposed to structural rearrangements leading to speciation in the genus Cricetulus.

Tumor cell lines T256 and T259 are subclonal in origin and exhibit the unique inverted tandem duplications. Cell lines derived from hyperplastic splenic follicles and/or bone marrow, such as T333, are aneuploid and lack inverted tandem duplications. The genetic consequences of the inverted tandem duplication encompasses such theoretical considerations as the source of redundancy and amplification of specific cistrons and possible steric hindrance or exclusion of antibodies due to the "dominance" of activated duplications of the genome.

Immunoblastoid transformants, when implanted into syngenic recipients, give rise to diffuse lymphomatous masses. Sera of tumor-bearing animals have excessive amounts of immunoglobulins and contain antibodies against nuclear antigens which cross-react with those of human T- and B-cells, and reticular neoplasms deriving from both species of hamsters. Comparative chromosome banding studies have disclosed several relationships between the karyotypes of the Chinese and Armenian hamsters, including the origin of the X chromosome in the latter species and its role in neutralizing extensive trisomic entities. An immediate subspecies of the Chinese hamster, Cricetulus barabensis barabensis (2n=20), bears the identical karyotype, except for the Robertsonian fusion of chromosomes #6 and #7 (6:7). The latter chromosome is also present in the Armenian hamster as the second largest metacentric.

The principle feature is the prominent role played by chromosome #1 of the Chinese hamster (or its equivalence) in events leading to the origin of three major chromosomes in the Armenian hamster (#6, #7, and X). Chromosome #1 of the Chinese hamster features two highly sensitive breakage sites which give rise to marker chromosomes in neoplasms and site-specific lesions when cultured cells are exposed to virus and chemical agents. Recent studies have disclosed that these same sites were implicated in the course of speciation leading to the migratorius subgroup of the genus Cricetulus.

The chromatin segments just distal to these sites on chromosome #1 are implicated in the formation of: (a) inverted tandem duplications; (2) chromosomes #6 and #7 of the Armenian hamster; and (c) the X chromosome of the Armenian hamster. Chromosome banding analyses have revealed 33 percent of the Chinese hamster genome (or its equivalence) was implicated in the formation of chromosomes #6, #7, and the X chromosomes of the Armenian hamster.

This configuration led to a 9 percent increase in the Armenian hamster genome. The surplus (trisomic) DNA is equivalent to 45 percent of the length of chromosome #1 in the Chinese hamster. It is represented in the Armenian hamster as the genetically-inactivated late-replicating segment associated with the X chromosome. The immediate (obligatory) inactivation of a lengthy trisomic segment following translocation to the X chromatin illustrates the vital role played by the sex mechanism in restoring the balanced diploid state to the newly created genome. Consequently, a major source of extraneous or repetitive DNA noted among related species may reflect this obligatory heterochromatinization of autosomal trisomies.

Approximately 84 percent of chromosome banding sequences in both species have remained intact linearly, while some 5 percent was implicated in a pericentric inversion forming chromosome #5 of the Armenian hamster. The remaining 11 percent of the chromatin is shared by the small metacentric-acrocentric chromosomes, #8-#10 and #8-#10, which display features suggestive of pericentric inversions.

Future comparative studies hopefully will reveal additional sites which express nonrandom breaks and reunions, their relationships with tumor progression and, possibly, overt functional expressions of reticular cell neoplasms.

Studies on Chemically-induced Nondisjunctions

Dr. Zsolt Harsanyi (Cornell University Medical Center) has begun studies on drug and chemically induced chromosomal aberrations (nondisjunctions), using the eukaryotic form of the fungus Apergillus nidulans. The versatility of the diploid state proved it to be a more favorable candidate than the haploid for these studies. The former gave rise to viable hyper- and hypodiploids, as well as to hyperhaploids, which were the only viable products of haploid nondisjunction. The diploid tester strain was constructed to carry the following 21 markers on eight pairs of homologous chromosomes (homologues indicated in parentheses; * indicates recessive auxotrophy): yellow conidia, p-aminobenzoic acid*, adenine*, adenine suppressor (IA); adenine* (IB); acriflavine resistance (IIA); white conidia, nitrite* (IIB); phenylalanine* (IIIA); thiosulfate* (IIIB); pyridoxine* (IVA); methionine* (IVB); lysine* (VA); nicotinic acid* (VB); thiosulfate* (VIA); lactose utilization (VIB); nicotinic acid* (VIIA); choline* (VIIB); riboflavine* (VIIB); riboflavine* (VIIIA); and chartreuse conidia (VIIIB). The strain was examined for its stability, measured in terms of the spontaneous chromosome alterations arising during mitosis. The types of spontaneous chromosome changes recovered from random platings were: (1) mitotic cross-overs, (2) nondisjunctional diploids, (3) aneuploids, (4) haploids, (5) translocations, and (6) recessive and dominant lethals, some of which were conditional. Initially all markers were examined for all segregants until experience permitted identification of specific types of segregants. Once the aberrant sergregants could be recognized and classified, the conditions for exposure were examined.

Quantitation could be carried out most readily with resting conidia, but treatment with known mutagens, formaldehyde (0.05 percent) and

p-fluorophenylalanine (1 percent), demonstrated only refractory conidia. Hence all treatments were carried out on germinating conidia.

Because germination rates differ when various compounds are added to the basic test medium, exposure to such compounds could not be carried out according to absolute time periods. Fluorescent staining of nuclei with acridine orange showed that regardless of physiological conditions, 97 percent of the uninucleate conidia became binucleate by the time the length of the germ tube equalled the diameter of the conidium. Hence such germination could be taken as the completion of the first mitosis. A high degree of synchrony was further achieved by brief nutrient starvation; previous trials with temperature shifts were actually more disruptive to replication. This synchrony was achieved optimally at 37°C; at the lower temperatures the percent of asynchronous hyphae increased to as much as 45 percent (optimum-6.3 percent).

The drug p-chlorophenylalanine was the first unknown to be tested. Unlike its structural analogue, p-fluorophenylalanine, there was no significant increase in nondisjunction at concentrations as high as 3 percent (2.9 percent aneuploids). Other compounds are now being tested.

In addition to mitotic nondisjunction, studies on potential mutagens and meiotic nondisjunction were initiated. Balanced heterokaryons were constructed carrying the same 21 markers as the diploid strain. Upon transfer to test media the heterokaryons did not form cleistothecia, the meiotic fruiting structure; hence growth conditions were varied. At present a medium with 0.3 percent urea as the nitrogen source appears to show greatest promise.

Studies on Clastogenic and Banding Effects of Metals

Dr. Jack Schubert (University of Pittsburgh) has initiated studies to determine the clastogenic and chromosome banding effects of a number of heavy metals. He has been successful in developing, for the first time, a technique for preparing bone marrow cells from the rat which have numerous advantages including: (1) no colcemid for arresting cell division is required, thus eliminating its distorting effects or possible interaction with metals; and (2) chromosomes from the same animal can be obtained at intervals during its lifetime. Aside from the clastogenic effects of metals, their effects on the banding patterns within individual chromosomes is being determined.

Physical Factors

There is growing concern about the effects of noise, microwaves, laser radiation, light and other physical factors on human health. Though some studies are underway and others are being supported when found to be of sufficiently high quality, the many ramifications of physical factors on health remains a relatively little studied area.

Heat

Dr. Pharo Gagge and his colleagues (John B. Pierce Foundation) have continued an interdiscriplinary study of man's response to his thermal environment. Predictive psychorometric charts for comfort and heat tolerance have been developed for three activity levels--sedentary, light work, and medium work. The chart takes into account factors such as clothing insulation, air movement, barometric pressure, sweat evaporation, and temperature.

The significance of recent findings and practical applications in human health include: Improved knowledge of the heat and transfer coefficients as they apply to the total heat exchange between clothed and unclothed subjects and the thermal environment. This makes it possible to develop psychrometric charts that can actually predict man's thermal comfort, the probable zone of evaporative regulation, and various levels of heat strain. It is possible to develop and set standards for heating, ventilating and air conditioning in residential and work spaces based on observed thermal sensations, comfort perceptions, resulting behavioral responses, and observed incidence of heat aggravated illnesses. With a better understanding of exercise and heat acclimation on both sweat and peripheral skin blood flow, the use of clothing insulation, of spot air movement for cooling and of spot radiant heating can be optimized in recommending protection of the working man from heat stress.

Continuation of work on physiological adaptation of women to heat stress by Dr. Barbara Drinkwater et al. (University of California) has been concentrated on effects of age and cardiovascular fitness levels on response to hot environments and an acclimatization of females to heat. Work with older women has opened up some new areas for investigation. A number of females have been found who can cope with severe heat stress with no apparent physiological or psychological difficulties within the limits of the experimental conditions. Age is not a factor since these females range in age from 14 to 44 years. Attempts will be made to match this group with another group of women who appear to be unusually sensitive to heat exposure and to isolate those factors which discriminate between the two groups. In order to obtain an adequate sample, this will be an ongoing study over the next two years.

The results of the study on intermittent work (75 percent $V_{O2\ max}$) in the heat have suggested further avenues for research in this area. Briefly, it was found that the physiological response is mediated primarily by the metabolic requirements rather than the ambient temperature. Using a work-to-rest ratio of 6 to 20 minutes, the women were able to return to a resting metabolic level between work bouts even though body heat content continued to climb during the recovery periods. Since most jobs in hot industries involve intermittent work, this line of investigation will be pursued by altering the work/rest time ratios as well as the level of physical work. Since the women's responses in the experiment just completed were independent of the more commonly used Heat Stress Indices, further investigation along these lines seem warranted.

Laser Radiation

Studies involving laser radiation are being carried out by Dr. Karl Weiss at Northeastern University in Boston. Laser radiation has potentially significant therapeutic applications, but it is also a health hazard. Dr. Weiss' research is concerned with understanding the mechanisms and consequences of laser excitation at the molecular level. A satisfactory laser has been designed specifically for multiphoton experiments with flavins and visual pigments considered to be of physiologic importance. In experiments with flavins, it was found that alloxazine, lumichrome, and riboflavin all give rise to fluorescence when subjected to pulsed laser radiation at 694 nm, whereas none show absorption in ordinary light. The experiments demonstrate that laser radiation can produce radicals from natural components which do not normally form as a result of other types of irradiation. It is suggested that such radicals may constitute the basis of laser radiation damage in cells.

Work was recently begun on the laser photolysis of visual pigments and related compounds. This should provide important insight into potential effects of lasers on rhythmic phenomena in view of the known role these pigments play in biological rhythms.

Light

In a basic study of physical factors on the endocrine system, Dr. Richard Wurtman and his associates at Massachusetts Institute of Technology have continued to explore the influence of light on hormonally-controlled processes in mammals. The studies are concerned with the basic molecular events through which neuroendocrine effects are initiated and carried out. Their findings constitute probably the first available information on the action spectrum for any nondermatological or non-visual effects of light on intact animals. They indicate that the photopigement which mediates the pineal response to light, and perhaps other neuroendocrine effects, is rhodopsin or a similar compound. Since rhodopsin is the photopigment of rod cells, and rod cells are the only photoreceptor cells in the rat retina, the data suggest that the separation of the photic input into that concerned with vision and that responsible for other physiological effects occurs not within the eye but solely within the brain. Thus retinal pathology that occurs in continuous light does not block the ability of the eye to function as the portal of entry for at least that portion of photic input concerned with neuroendocrine functions. Further studies on the pineal enzyme hydroxyindole-O-methyl transferase (HIOMT) have been carried out. HIOMT enzymes from the retina and harderian gland, which are concerned with control of the daily rhythm in body temperature, have been characterized with respect to biochemical properties and compared with the pineal enzyme. It appears that the retinal and pineal enzymes are similar but differ from the harderian gland enzyme.

In addition to control of body rhythms through the endocrine system, light was shown to have an influence on the acute toxicity of certain natural toxins, such as aflatoxin and monocrotaline (hepatotoxins). In the case of aflatoxin, either light or riboflavin will enhance acute toxicity, and when the two are combined there is further enhancement. On the other hand, carotene appears

to inhibit aflatoxin hepatoxicity, with or without light. Monocrotaline toxicity is inhibited by light, and light plus riboflavin has a marked protective effect against its action. These results indicate the complex nature and the potential importance of light in the action of sensitizing food ingredients in biological systems.

Dr. Frederick Urbach and his colleagues (University of Pennsylvania) have continued work on several aspects of the biological effects of light. Concern has grown recently about possible alteration of the earth's atmosphere with resultant changes in the quality of light reaching the earth's surface. In addition, there is concern about the plethora of photoactive chemicals to which the population as a whole is being exposed.

New equipment for monitoring, metering and measuring irradiation of experimental animals and man has been developed. This provides heretofore unavailable capabilities for diagnosis of light-induced diseases of man, for treatment of certain diseases and for quantitative and qualitative studies of various spectra on tumorigenesis and other photobiologic phenomena.

Skin cancer can be induced reliably in animals by use of the new equipment and techniques. The techniques also provide predictive capability with respect to the consequences of diminishing atmospheric ozone and the interaction of light and chemicals in photocarcinogenesis of the skin. For example, it was discovered that delayed hypersensitivity plays a protective role in visible light and UV-induced skin cancer. This may be of great significance because it suggests that stimulation of the immune system or other protective mechanisms can prevent or reduce the incidence of skin cancer and that immunotherapy might be of real value.

The complicated photochemical transformation of environmental carcinogens is being studied. It was found that different photoproducts are produced by light at different wavelengths. Some of the products are highly carcinogenic, others noncarcinogenic. The chemistry of the products is being investigated.

The problem of side effects in the phototherapy of hyperbilirubinemia in infants is being investigated. New spectrally narrow, highly active lamps have reduced treatment time and increased the rate of bilirubin destruction. However, serious side effects are possible, such as damage to unprotected retinas (particularly in the presence of oxygen), reduction of riboflavin, and changes in biological rhythms. These are being investigated to find ways to avoid unwanted reactions.

Photosensitivity. Dr. Leonard Harber (Columbia University) has continued studies designed to recognize and assess environmental agents which cause adverse cutaneous photosensitivity reactions in man, to develop biochemical and biophysical tests of photosensitivity diseases, and to determine the mechanisms of adverse reactions to light. Continued clinical study of patients with known photosensitivity diseases resulted in the recognition and subsequent characterization of differences in the emission spectra of porphyrins associated with lead poisoning and erythropoietic protoporphyria. Contact photosensitivity to "Dowicide 32," chloro-2-phenyl phenols was induced in 21

of 34 guinea pigs. Variations in the incidence of induction and magnitude of elicitation of effects by these compounds at different temperatures and relative humidities can now be compared.

A ten-year evaluation of erythropoietic protoporphyria and the response of 32 selected patients to beta carotene has been completed.

The characterization of porphyrins in lead poisoning showed them to contain a zinc protoporphyrin strongly bound to globin at empty heme sites. This porphyrin has a different fluorescence emission spectra than found in erythropoietic protoporphyria.

Passive transfer of contact photosensitivity to 3 3' 4' 5-tetrachlorosalicylanilide was accomplished with a splenic lymphocyte suspension of 3×10^8 cells in one of two attempts in guinea pigs tested. Three attempts at obtaining a positive lymphocyte stimulation test were unsuccessful.

Research in photosensitivity was active during the past year in aiding the FDA in its efforts to have 3, 4', 5-tribromosalicylanilide removed from household soaps. Previous studies in guinea pigs and man done under the aegis of this grant had indicated its mechanism of photosensitization as well as the dangers of its photosensitizing potential.

Studies were continued on the use of beta-carotene as an oral agent in the treatment of erythropoietic photoporphyria. Although still in the investigational stage, this compound appears to be an excellent therapeutic agent and is approaching international acceptance. Tests showed the compound to be effective in 49 of 53 patients studied. Efforts are being made to find other non-toxic systemic agents that will protect against 300 and 360 nm radiation.

Noise

Dr. Robert Hamernik (State University of New York Medical Center) has continued studies on the auditory effects of combined impulse and continuous noise, which was shown previously to cause large hearing losses and hair cell damage in the chinchilla. The studies show that chinchilla exposed to 50 impulses of 40 usec A duration at 137 to 147 dB developed large values of TTS. Hair cell damage ranged from 2 to 5 percent OHC losses in the 137 dB group and almost complete loss of all OHC and large losses of IHC in the 147 dB group. The conclusion from the experiments is that 137 dB impulses superimposed on a 95 dB SPL 2-4 kH_z band of noise for one hour is definitely unacceptable exposure for the chinchilla and constitutes a significant auditory hazard. More work has to be done to generalize such results to man.

Similar experiments involving exposures with markedly different noise spectral content gave similar results, producing larger values of TTS than would be expected from the simple addition of acoustic power from the mismatched spectra.

Synergism between noise and drugs. A number of recent experiments have shown a synergistic interaction of noise and ototoxic drugs. The most commonly used ototoxic drug is the family of salicylates. Therefore, a series of experiments were conducted to see if there is a potentiating effect of sodium salicylate with the traumatic effects of noise. Several classes of noise were used: short duration--high intensity; long duration--lower intensity; and impulse noise. Chinchilla were injected with 400 mg/k of sodium salicylate and then exposed to one of the three noises. For all three classes of noise there was no evidence of any interaction between noise and the drug. Each of these experiments demonstrated that the agent which produced the greater TTS would dominate the overall audiometric results. The practical conclusion is that salicylates seem to present no additional hazard to individuals working in noise environments. The theoretical implications of the experiments may be equally important. While other ototoxic drugs appear to interact with traumatic noise sodium salicylate typically produces only temporary hearing loss and it does not interact with noise.

Since the diagnostic power of quiet-threshold audiometry is limited, considerable effort has been directed toward developing other measures of auditory acuity in the chinichilla. One such effort is the use of the startle-response, avoidance-conditioning technique. Results of the study show that with primarily outer hair cell lesions, the integrative behavior may be a more reliable measure of cochlear pathology than simple threshold tests.

OFFICE FOR INTERAGENCY PROGRAMS
Summary Statement

During the past year, this Office has played the lead role on behalf of the Office of the Director, NIEHS, principally in two areas: US-USSR Cooperation and Energy-Related Research.

US-USSR COOPERATION

Collaboration between Soviet and American environmental health scientists is being carried out under the aegis of two cooperative agreements between the United States and the Soviet Union. Under the Medical Science and Public Health Cooperative Agreement, scientists from both countries are committed to regular, long-term collaboration in the areas of heart disease, cancer, arthritis, influenza and acute respiratory diseases, and health problems associated with the environment. The Director, NIEHS, is U.S. Coordinator for the environmental part of the Health Agreement.

1976 was the fourth year of formal cooperative efforts in environmental health research between the US and USSR. The first year was concerned largely with establishing working relationships and agreeing on areas for joint study. Joint research efforts were initiated in the second year of the Agreement and involved exchange visits between scientists of both sides. The research results developed during the second and third year of cooperative research were presented by American and Soviet scientists participating in the Agreement at a symposium in Riga, Latvia, in December 1974. The results of this symposium were published in 1976 in both the United States and the Soviet Union.

Scientific results from the cooperative research plan for 1975-1976 will be presented at a second joint symposium to be held in the United States in December 1976. Current research efforts are divided into three problem areas which seek to explore the methodological basis for assessing the biological effect of chemicals which might be inhaled, or introduced orally, or both simultaneously. During the meeting of the US-USSR Joint Committee for Health Cooperation in October 1975, a fourth problem area was added aimed at exploring the methodological basis for assessing the biological effect of physical environmental factors.

By the end of 1976, over 25 scientific papers will have been published by American and Soviet scientists on environmental health research conducted under the auspices of this agreement.

During 1976, a Russian-English Glossary of Environmental Health Terms was completed.

The Agreement on Cooperation in the Field of Environmental Protection between the US and USSR is aimed at addressing the most important aspects of problems of the environment, including the study of pollution and its effects on the environment. A number of agencies participate in this Agreement which is under the general direction of the Administrator, EPA. The Director, NIEHS, serves as HEW representative to the Environmental Protection Agreement and as Co-Chairman of the working group for one of the areas of the Agreement, the Biological and Genetic Effects of Pollutants. Effort in this area has been focused on the mutagenicity of environmental contaminants, the toxic effects of heavy metals in the environment, the application of epidemiological methods to the study of environmental pollutants, and psychophysiological effects of environmental pollutants. A new topic area added in 1975 seeks to assess the potential environmental health hazards associated with oil shale by-products.

During 1975-1976, program activity under this Agreement consisted of exchange of information and visits by scientists of both sides including the Second Joint Workshop on "Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis" held in Dushanbe in the USSR.

Energy-Related Research

As part of the high priority accorded by the Federal Government to solving the energy problems facing the nation, the Director and staff of NIEHS are involved in a variety of activities to elucidate the potential adverse health impacts of energy technologies.

During the summer and fall of 1974, an OMB/CEQ task force met to identify needed research on the potential adverse health and environmental problems associated with energy use. As part of the report of the Interagency Working Group on Health and Environmental Effects of Energy Use published in November 1974, the Health Sub-Group, co-chaired by the Director, NIEHS, recommended that research be pursued in several areas. As a result of the work of this task force, NIEHS was assigned the responsibility, and given additional funds, to develop research programs to address a number of important energy-related questions, including the development of physiological indicators to estimate damage to man; the determination of mechanisms of incorporation, metabolism, deposition and turnover of hazardous agents; the determination of the relationship of metabolism and fate and the toxicology of particulates and organic compounds; the determination of the dose-effect relationship for mutagenic effects; the development and testing of models for extrapolation of cellular and animal data to man; the identification of damage to cells and cell components; and the determination of immediate and long-term effects of critical pollutants on selected ecosystems and organisms in the marine environment.

During 1975-1976, NIEHS initiated a variety of new projects focused on the problems indicated above. In January 1976, NIEHS co-sponsored, along with NIOSH, EPA, and ERDA, a retreat for about 150 scientists working on energy-related health problems. NIEHS staff participated with staff of other agencies in various meetings devoted to the review of energy-related health problems.

OFFICE OF PROGRAM

OFFICE OF PROGRAM
Summary Statement

During Fiscal Year 1976 the Office of Program continued its activity of surveillance of potential environmental health hazards resulting from technological developments. The staff was expanded slightly. It consists of a Chemical Engineer, a Pharmacologist, a Neurobiochemist, and a consultant with expertise in toxicologic and legal situations, besides its Associate Director.

Although most of the assessments of toxicologic significance are made by asking questions based on the properties and uses of important chemicals, the opposite approach was used; starting with disease and adverse effects on specific organs or tissues and searching for the causative chemicals in the world literature. This procedure tends to assure that toxicologic effects -- not in the limelight or the primary effects -- may not be overlooked.

Regarding recently detected toxic chemicals, interest in them has been maintained through the year. Members of the staff have searched for important chemicals, structurally related to vinyl chloride which may still be in common use, as their similarity in toxic action has as yet not been established. An effort will be made to bring these structural relationships to the attention of scientists without implying that they necessarily must be toxic in a similar fashion. The Institute is prepared to hold an International Conference on the toxicity of vinyl chloride-related compounds to be carried out next fiscal year under the auspices of the NAS and contacts with international authorities in that field have been made to assure adequate participation.

Interest has also been maintained on the toxicity of fire retardants for fabrics where the chemical may make contact with the skin under normal use, but where other toxic effects may be anticipated under conditions of pyrolysis or fire for which the materials have been developed.

The potential risk to man and the environment from the slow depletion of the stratospheric ozone layer by the large-scale use of fluorochlorinated hydrocarbons has been one of the activities of our pharmacologist in this Institute's participation on the Federal Task Force on Inadvertent Modification of the Stratosphere (IMOS) which continued this FY. A study of other chemicals which may have similar effects if they reach the stratosphere has been attempted.

With regard to technological developments in energy procurement for the immediate future, the utilization of methanol as a readily available fuel for automobiles has been proposed by industry and a staff member has been active, attempting to educate physical scientists on the potential health hazards that may be encountered. In the literature the transformation of methanol to

hydrocarbon fuel has been recorded which would avoid some of the anticipated hazards. Hydrocarbons however are also toxic and their toxicity has been studied. Their oxidation to ketones may be responsible for neurotoxicity which deserves further study, as not all aliphatic ketones are similarly neurotoxic.

As the result of his participation in an NSF sponsored activity regarding the ranking of industrial chemicals for their environmental and health impact a member of the staff advised the Institute on high priority chemicals for which inadequate information exists regarding their mutagenic or teratogenic properties. He will participate under the chairmanship of the Associate Director for Program in the deliberations of the newly established NIEHS priority assessment committee for testing adverse effects of chemicals.

During the past year, the Safety Officer has expended considerable effort in implementing the Institute's Safety Program. A medical surveillance program has been established and is using the facilities of the Duke University Medical Center. The primary method of identifying Institute personnel for this program is the Hazardous Chemical Use Protocol. A program on the use of fire extinguishers has been started and will be continued so that all personnel will gain necessary experience in the proper use of fire extinguishers. The Safety Officer has participated on the DHEW Committee for Establishing Safe Laboratory Procedures for Handling Chemical Carcinogens. Activities of this Committee have been directed towards establishing a laboratory safety course for laboratory personnel on the handling of chemical carcinogens. In addition, this Committee is engaged in providing safety monographs on chemical carcinogens which will be practical working guides for normal handling of these materials, what to do in case of an accident, and how to dispose of material remaining after the completion of the program. Better methods of waste disposal of residual chemicals from experimental programs continues to be a high priority concern. Unused or unwanted chemicals are currently sent to a sanitary landfill, but efforts continue in finding methods to chemically or thermally destroy waste chemicals from experimental programs. Progress has been made in maintaining clear corridors in laboratory buildings and in the handling of compressed gases. The Safety Officer is aided in general surveillance of facilities and operations by his assistant, Curtis Carroll, and by building monitors.

Since the NIEHS has been chosen to become a WHO-Collaborating Center several programs have been carried out involving the Associate Director for Program or his staff. In September 1975 the Institute was visited by Dr. Pavlov, the Assistant Director General, and Dr. Dieterich, the Director of Environmental Health of WHO, to become familiar with the Institute and its senior staff before declaring it a WHO-Collaborating Center.

For this collaboration which has taken place on a less formal basis already for several years, the Associate Director for Program has prepared a chapter for the WHO Manual on the Evaluation of the Toxicity of Chemicals on "Modulating Factors" and participated in a conference in Geneva where all chapters were reviewed by all authors participating in writing the manual. He also participated in the review of one of the International Environmental Health Criteria Documents, i.e. on polychlorinated biphenyls, which was held in Copenhagen,

Denmark, and wrote four monographs on specific natural carcinogens for the IARC Conference on Natural Carcinogens which he attended in Lyon, France.

Collaboration with WHO was brought to fruition in the jointly sponsored WHO/NIEHS Symposium on Potential Environmental Health Hazards from Technological Developments in Rubber and Plastics Industries which was held at the Institute in March, 1976, bringing together experts from the U.S.A. and ten foreign countries. The proceedings of this conference will be published in the NIEHS' Journal, ENVIRONMENTAL HEALTH PERSPECTIVES. This office will also assist in another conference sponsored by WHO and the WHO-Cooperating Center in Sweden and supported by NIEHS on the Toxicity of Arsenic.

The Associate Director for Program has been invited by the German Research Community to give a talk on the "Possible Mechanisms of Combination Effects in Chemical Carcinogenesis" in a symposium initiated by the WHO on "Threshold Doses in Chemical Carcinogenesis" (Heidelberg). In a WHO-Euro sponsored conference, the Associate Director has been invited to talk about trace contaminants in water and food (Brussels).

The Associate Director for Program helped in the organization of the NIEHS-sponsored conference on "Human Health Effects of New Approaches to Insect Pest Control" and presented an introduction on this timely topic. He also participated as a speaker in the second NIEHS-sponsored conference on "Problems of Extrapolating the Results of Laboratory Animal Data to Man and of Extrapolating the Results from High Dose Level Experiments to Low Dose Level Exposures" talking about the "Biological Evidence for the Existence of Thresholds in Carcinogenesis".

In OSHA's Occupational Cancer Seminar, the Associate Director for Program gave an invited talk on "In vitro Test Systems for Carcinogenicity". On invitation he discussed "The Purity of Water and Its Use by the Food Industry" at a Conference on "Chronic Toxicity Testing and the Food Industry" initiated by Dr. Shubik and held at the Given Institute of Pathobiology.

The Associate Director for Program helped Dr. Schiller of this Institute in the planning of a conference on "Ethics and Human Value Implications of Science and Technology" which was sponsored by the W. Alton Jones Cell Science Center and where he gave a talk on "The Need for Rapid Assessment of Chronic Toxicity of New Chemials".

On request by the HEW Toxicology Coordinating Committee, the Associate Director for Program serves as Chairman of the Committee to Assess the Hazards to Health from Lead Compounds Originating from Auto Exhausts. He also serves on the HEW Laboratory Chemical Carcinogens Safety Standards Subcommittee.

The staff of this Office is heavily involved in helping HEW in the assessment of drafts on Effluent Limitation Guidelines and Environmental Impact Statements prepared by Federal agencies, particularly EPA, with emphasis on health hazards. This activity turned out to be the most time consuming and unrewarding activity.

The Associate Director for Program serves as consultant to the National Cancer Advisory Subcommittee on Environmental Carcinogenesis, which is an important function of considerable interest and relevance to this Institute.

As a member of the Committee on Biological Effects of Ionizing Radiation (BEIR) of the National Academy of Sciences and as a representative of NIEHS to the Medical and Biological Effects of Environmental Pollutants Committee (MBEEP), the Associate Director for Program advises and keeps informed on areas of concern to the NIEHS and NAS.

In order to facilitate completion of the Handbook of Environmental Physiology for the American Physiological Society, the Associate Director for Program took over as Associate Editor for retiring, Dr. Douglas H. K. Lee, and reviewed all submitted manuscripts for acceptance in the Handbook. He also remained active on the editorial board of Environmental Health Perspectives, the Journal of the National Cancer Institute, the Journal of Toxicology and Environmental Health, Environmental News Letters, and as a reviewer for other journals on request.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20001-04 ADP

PERIOD COVERED
July 1, 1975, to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Variations in the Response of Rats to Porphyrogenic Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hans L. Falk	Assoc. Dir. for Prog.	OP NIEHS
OTHER:	Sandra Jordan	Technician	OP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

None

SECTION

None

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Feeding hexachlorobenzene to rats is well known to produce porphyria, an effect which does not decline readily after feeding the chemical has been stopped. The difficulties in elimination of hexachlorobenzene is taken as the reason why the porphyria lasts so long after feeding has been discontinued. Individual rats on this diet show great variation in the magnitude of the porphyrin excretion, the interval to maximum porphyrin elimination and the decline of porphyrin elimination which may not reach base line and may take months to reach a steady state level. On the other hand some animals do not respond to feeding hexachlorobenzene with urinary porphyrin excretion, which is an unusual observation.

PROJECT DESCRIPTION

OBJECTIVES: To study the porphyrogenic response of rats to chlorinated hydrocarbons given in the diet, to assess the severity of the response, the duration and the recovery following cessation of treatment. To understand the factors involved in producing the observed variations in response.

METHODS EMPLOYED: Young female rats were given hexachlorobenzene in the diet for specific time intervals. Urinary elimination of copro- and uroporphyrin was measured spectrophotometrically. The chemical would be re-introduced into the diet and the effects on urinary porphyrin excretion would again be measured. Weight gain of the animals is monitored and at the end of their life histopathological examination is given to the animals.

MAJOR FINDINGS: Feeding hexachlorobenzene to rats is well known to produce porphyria, an effect which does not decline readily after feeding the chemical has been stopped. The difficulties in elimination of hexachlorobenzene is taken as the reason why the porphyria lasts so long after feeding has been discontinued. Individual rats on this diet show great variation in the magnitude of the porphyrin excretion, the interval to maximum porphyrin elimination and the decline of porphyrin elimination which may not reach base line and may take months to reach a steady state level. On the other hand some animals do not respond to feeding hexachlorobenzene with urinary porphyrin excretion, which is an unusual observation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In order to assess variability in human responses to toxic chemicals it is imperative to understand all the variables that may be at play. In this case different responses have been observed in groups of caesarean-derived rats of the same sex exposed to the same regimen which showed alteration in response to subsequent exposure to hexachlorobenzene for which no reasons are readily available.

PROPOSED COURSE: We shall attempt to control as many variables as possible in these experiments of very long duration and pay attention to aging, enzyme induction inhibition, and other factors to understand the effects. We shall attempt to prevent prophyria with the timed exposure of rats to phenobarbital and other chemicals prior to chlorinated hydrocarbon feeding.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20002-04 ADP

PERIOD COVERED

July 1, 1975, to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Technology Surveillance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Warren T. Piver	Chemical Engineer	OP NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for Prog.	OP NIEHS
	Herbert S. Posner	Pharmacologist	OP NIEHS
	Terri Damstra	Biochemist	OP NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH

None

SECTION

None

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this program are: 1) to stay abreast of present and developing technologies related to the production and use of chemicals; 2) to examine the sources, rates of release, methods of transport, and potential levels of exposure to hazardous chemicals; 3) as a result of objectives 1 and 2, to suggest research needed to provide a firmer scientific basis for regulation of hazardous materials; and, 4) to suggest modifications in chemical plant design, product design, use pattern, or disposal practice to minimize adverse environmental effects.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this program are: 1) to stay abreast of present and developing technologies related to the production and use of chemicals; 2) to examine the sources, rates of release, methods of transport, and potential levels of exposure to hazardous chemicals; 3) as a result of objectives 1 and 2, to suggest research needed to provide a firmer scientific basis for regulation of hazardous materials; and, 4) to suggest modifications in chemical plant design, product design, use pattern, or disposal practice to minimize adverse environmental effects.

METHODS EMPLOYED: The methods used in this program are: 1) literature review aimed at synthesis of available information on chemical processes, losses of material during processing, production and disposal of by-products, uses of manufactured chemicals, trends in usage, recognition of structurally similar chemicals, and biological investigations conducted on these chemicals; 2) membership on panels and committees concerned with environmental health aspects of chemicals; and, 3) continual upgrading and refinement of skills in chemical engineering science so that suggested modifications in processes and practices will reflect the latest knowledge in dealing with these problems.

MAJOR FINDINGS: A. The Trace Organic Chemicals in the Environment Panel sponsored by NSF developed a methodology for ranking industrially important chemicals. The final list was composed of 80 chemicals. Information which was obtained on these chemicals included stability constants, chemical reactivity data, mobility data, and toxicity information. Of the 80 chemicals, 66 had no data on mutagenicity and 78 had no information on teratogenicity. This information was passed onto the appropriate research managers at the Institute.

B. Reviews of EPA Effluent Limitation Guidelines and EIS for the Office of the Assistant Secretary for Health, DHEW, have shown that these documents have a consistent lack of understanding of rational pollution control strategy and environmental impact of proposed projects. Consistent suggested changes have been: 1) better characterization of waste streams; 2) segregation of problem wastestreams; 3) more attention to occupational health impact; and 4) more attention to solid waste disposal practices.

C. The particle size of PVC resins has been shown to be an important physical-chemical property which influences the amount of unreacted or residual monomer in PVC resins. Other important properties of monomer-polymer systems are monomer solubility and diffusivity in the polymer. A similar program has been initiated to define those properties of plastics which influence rate of migration of non-reactive chemical additives.

D. As a result of Federal legislation, the number of chemicals being used to retard fires is increasing. In many instances, the mechanism of retardation, the nature of the combustion products, and the effects of exposure to these substances is either unknown or poorly understood. Participation on an ASTM

Fire Hazard Rating Function Committee has revealed the paucity of information on the toxicology of combustion products of materials when subjected to heat and flame. Two main questions have arisen during this inquiry: the nature of the combustion products and how long it requires for these substances to render a person in varying states of health and consciousness, incapable of escaping the fire situation.

The residual effects to the survivors of such an exposure are very poorly understood.

E. A program of simulation of transport processes occurring in sanitary landfills and spent oil-shale disposal dumps has been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These surveillance and liaison activities will continue to provide necessary information about products and use patterns of chemicals, important environmental transport mechanisms, and development of methods to predict exposure levels for environmental chemicals. Such information can be used in planning research programs for the Institute.

PROPOSED COURSE: This work will continue and will expand into other areas of interest. This program will attempt to include more and more Institute personnel and representatives from other disciplines and background.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20003-03 ADP

PERIOD COVERED
July 1, 1975, to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Preventive Surveillance of Environmental Chemicals for Toxic Potential

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert S. Posner	Pharmacologist	OP NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for Prog.	OP NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Specific compound series monitored include aminimide and vinyl halide- and vinylidene halide-containing compounds. In the first instance, the compounds are of a relatively new structural class whose biologic activities prior to and after metabolism remain to be determined. In the latter instances, it remains to be determined what structures attached to the stated functional groups result in retention of biologic hazard, perhaps as known for vinyl chloride. The biologic hazards of methanol were reviewed including its context in the many proposed uses in the future. Participation on the IMOS (Inadvertent Modification of the Stratosphere) Task Force of the Council on Environmental Quality and the Federal Council for Science and Technology, and one of its subcommittees resulted in a report entitled, "Fluorocarbons and the Environment" and in a proposal for short- and long-term research on the biologic and climatic effects of potential ozone reduction in the stratosphere.

PROJECT DESCRIPTION

OBJECTIVES: The project develops and utilizes a variety of modes of preventive surveillance appropriate to NIEHS in the attempt to minimize possible toxic events in specific or general populations. The information developed relates, first to the compounds or physical agents being considered, and then as much as possible to related chemical, physical, or biological systems.

METHODS EMPLOYED: Searching of the literature, consultations with appropriate individuals, consideration of known structure-activity relationships, attendance at meetings, preparation of reports, discussions with those who might assist in further laboratory or theoretical considerations, feedback and dissemination of the appropriate findings, and preparation of manuscripts for publication.

MAJOR FINDINGS:

Aminimide compounds: The literature is being followed on this interesting, relatively new series of structures outlined in two previous reports. The compounds are inner salts of structure N^{+}-N^{-}-R. Two reports have now appeared indicating broad spectrum antibiotic activity for some and preliminary LD_{50} values in mice. These reports appear to be the first in the open scientific literature for biological activity other than plant growth inhibition.

Compounds with vinyl halide or precursor vinyl halide substructures: The relationship of structure to activity continues to be considered for compounds that contain the vinyl halide type substructure. Research suggestions are offered at appropriate junctures.

Methanol: The review prepared on the biohazards of methanol has now appeared. Opportunity was provided to review the Draft NIOSH Criteria Document for methanol and to provide information for use at a state hearing on commercial applications for methanol. Several groups conducting tests on gasoline-methanol blends have been approached in the attempt to gain early knowledge of the steps being taken to prevent the toxic hazards from developing.

Qualitative and quantitative aspects of structure-activity relationships: This field is being monitored for techniques that will help to predict similarities of activities between "apparently" dissimilar structures. Substructural analysis, pattern recognition, and side effect sorting are some of the techniques that will be useful.

Outside activities: Meeting attendance: Environmental Impact of Water Chlorination; Conference on the Atmosphere: Endangered and Endangering; Conference on Human Health Effects of New Approaches to Insect Pest Control; Conference on Potential Health Hazards from Technological Developments in the Plastic and Synthetic Rubber Industries; Effects of Antioxidants Toxicity; Association of Clinical Scientists; in addition to regularly attended Society meetings.

Outside activities: IMOS Task Force and its BACER Subcommittee: The Task Force on Inadvertent Modification of the Stratosphere published a document entitled, "Fluorocarbons and the Environment". The Subcommittee on Biological and Chemical Effects Research prepared two reports for the Federal Council on Science and Technology. These are now being considered and will form the basis for the Federally-sponsored short- and long-term research to clarify the extent of some of the effects.

Review of documents: A large number of documents prepared by EPA, NIOSH, and DOT have been reviewed for health issues.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The project identifies areas where research is needed in order to prevent or reduce toxicologic hazard from environmental chemicals or from sources of physical stress (the latter as in the IMOS considerations). It is primarily a program of preventive and early-warning surveillance. It is linked closely to research dealing with the scope of the problem, mechanisms involved, and preventive measures. It also takes into consideration potential means of diagnosis and therapeutics where these might be possible.

PROPOSED COURSE: The new findings of biologic and toxicologic importance for the aminimide compounds will be followed as they appear. The vinyl halide situation will be followed closely and a manuscript in preparation will be completed. Additional channels will be sought for appropriate consideration of the hazards of methanol so that, if methanol is to be used in some of the proposed situations, its hazard can be minimized. The qualitative aspects of structure-activity relationships will be considered further. Meeting attendance, participation on special projects, review of documents and manuscripts, and responses for information will be continued.

PUBLICATIONS

Posner, H.S.: Biohazards of methanol in proposed new uses. J. Toxicol. Environ. Health 1: 153-171, 1975.

Bus, J.S., Preache, M.M., Cagen, S.Z., Posner, H.S., Eliason, B.C., Sharp, C.W. and Gibson, J.E.: Fetal toxicity and distribution of paraquat and diquat in mice and rats. Toxicol. Appl. Pharmacol. 33: 450-460, 1975.

Fluorocarbons and the Environment. Report of the Federal Task Force on Inadvertent Modification of the Stratosphere (IMOS). Council on Environmental Quality, Federal Council for Science and Technology, Washington, D. C. U.S. Government Printing Office, June 1975, 109 pp.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 20004-02 ADP

PERIOD COVERED
July 1, 1975, to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Technological Surveillance of Potential Neurobehavioral Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terri Damstra	Biochemist	OP NIEHS
OTHER:	Hans L. Falk	Assoc. Dir. for Prog.	OP NIEHS
	Warren T. Piver	Chemical Engineer	OP NIEHS
	Herbert S. Posner	Pharmacologist	OP NIEHS

COOPERATING UNITS (if any)

Institute-wide

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range objectives of this project are to 1) provide information on the neurological and behavioral effects of chemicals that may accumulate to critical levels following long-term low-level exposure and 2) to assess the practicality and validity of using neurobehavioral tests as early indicators of toxicity and surveillance "tools".

PROJECT DESCRIPTION

OBJECTIVES: 1) To determine whether exposure to a large variety of chemical agents from both existing and developing technologies may result in long-term neurological and behavioral consequences. 2) To assess the availability, validity, and utility of animal test systems which are used to reveal such neurobehavioral toxicity. 3) To evaluate methods for monitoring neurobehavioral effects in exposed humans. 4) To suggest research programs necessary to establish a firm scientific basis for using neurophysiological and behavioral tests as early warning indicators of toxicity.

METHODS EMPLOYED: Literature review aimed at integrating the available information on neurobehavioral toxicity. Attendance at meetings and membership on committees concerned with the neurological and behavioral effects of various agents. Reviews of documents and manuscripts; preparation of reports; and consultations with scientists from other Government agencies, industry, and academia.

MAJOR FINDINGS: Human populations are exposed to a number of toxic agents which are reported to affect the central nervous system at the threshold concentration or at concentrations two or tenfold above the recommended level. These agents range from toxic gases, heavy metals, chlorinated hydrocarbons, organophosphorus insecticides, industrial solvents, and household products, to a large number of drugs such as tranquilizers, stimulants, hormones, and anaesthetics. These may affect the rate of neurobehavioral development, sensory processes, motor control, emotionality, cognitive functions, and social behavior. Prenatal and neonatal exposures may have neurobehavioral effects which are not expressed until much later in the life span of the organism. The old and "stressed" organism may also exhibit enhanced susceptibility.

A review of agents whose main toxic effect is peripheral neuropathy indicates that many widely used industrial solvents and food flavorings (e.g. methyl-n-butyl ketone) may cause irreversible peripheral neuropathy at dose levels where other systems are not affected. At the present time, very little is known about the mechanism of toxicity of these compounds. Continuing surveillance of these compounds is critical.

It is very apparent that there is at present a lack of rational criteria for selecting suitable experimental procedures to determine the neurobehavioral effects in both animal test systems and human populations. Many of the studies reviewed are difficult to evaluate. For example, an extensive review of the literature on the psychological and neurological sequelae of low-level lead exposure in children, indicates that no definitive conclusions can be made as to the reported association between low doses of lead and "minimal brain dysfunction" in children.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The literature surveillance and liaison activities with outside scientists will identify the research programs necessary in order to assess the neurobehavioral effects of environmental chemicals.

PROPOSED COURSE: Literature surveillance, meeting attendance, participation on committees, and reviews of documents will continue and expand. An attempt will be made to include more Institute personnel.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 20005-01 ADP

PERIOD COVERED
July 1, 1975, to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chronic Lead Exposure: Neurochemical Assessment of Developmental Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Terri Damstra	Biochemist	OP NIEHS
OTHER:	Carol A. Kimmel	Senior Staff Fellow	ETB NIEHS
	L. D. Grant	Associate Professor	UNC

COOPERATING UNITS (if any)

None

LAB/BRANCH
None

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, N. C. 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range goals of this project is to elucidate the biochemical basis of lead toxicity in the developing central nervous system. The initial objectives are to determine whether chronic lead exposure:

1) is accompanied by alterations in the developmental patterns of the neuronal enzyme, Acetylcholinesterase; and the glial enzyme Butyrylcholinesterase.

2) inhibits brain adenyl cyclase, an enzyme closely associated with synaptic transmission.

PROJECT DESCRIPTION

OBJECTIVES: The goal of this project is to investigate the biochemical basis of lead toxicity in the developing central nervous system. The initial approach will be to determine whether chronic lead exposure in rats:

a) is accompanied by alterations in the developmental patterns of the neuronal enzyme, Acetylcholinesterase, and the glial enzyme, Butyrylcholinesterase.

b) interferes with brain cyclic AMP metabolism, by inhibiting adenyl cyclase, an enzyme closely involved in synaptic transmission, and shown to be inhibited by low levels of lead in vitro in mammalian brain tissue.

METHODS EMPLOYED: Rats will be chronically exposed to lead acetate via the drinking water (0.5 to 250 ppm) from weaning through mating, gestation, and lactation. At various stages of development, brain fractions will be prepared and the above enzyme activities assayed using standard published biochemical techniques. Blood and bone Pb concentrations will also be determined. The above neurochemical parameters will be evaluated in conjunction with behavioral and developmental assessments.

MAJOR FINDINGS: None. This project has only recently been initiated (February 1976).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The developmental toxicity of lead due to chronic exposure is of great concern due to its wide-spread low-level contamination. Although lead influences the activity of several enzymes, no biochemical basis for lead toxicity in the central nervous system has been clearly established. An analysis of the above enzyme systems may elucidate some of the factors involved in the toxic manifestations of lead in the nervous systems.

PROPOSED COURSE: To carry out the above outlined research.

OFFICE OF THE SCIENTIFIC DIRECTOR

OFFICE OF THE SCIENTIFIC DIRECTOR
Summary Statement

Direction of the Intramural Research Programs at NIEHS has been reorganized (to conform more closely with other NIH Institutes) by creating the Office of Scientific Director. To this office now report the Branches of Environmental Mutagenesis, Biometry, Toxicology, Pharmacology, and Biophysics. The Division of Research Resources contains both support and applied research programs; and, close collaboration between the Scientific Director and the Director of the Research Resources Programs is maintained. The new organization will provide for the kinds of ongoing management and monitoring of programs needed to assure quality, relevance and collaborative efforts with minimal overlap and duplication. The new organization will also stress planning for new research programs and expansion of present research programs so as to have a broadly-based extramural research effort in place and able to expand relatively quickly as our new building is finished in 1979 or 1980.

As part of the efforts at quality control, the various laboratory chiefs and program directors meet regularly with the Scientific Director to go over ongoing and proposed research. Program review of the entire Institute research effort occurs at several occasions: (1) the annual program review--conducted by the Scientific Director and laboratory or branch chiefs; (2) the Board of Scientific Counselors' Meetings (twice a year); (3) the annual visit of Drs. Stetten, Chen, and Talbot.

The annual program review covers all research programs and took three full days this year (in January). The Board of Scientific Counselors reviews the entire program over a period of three years--each meeting looks at one laboratory or a program at a time. The annual meeting with Dr. Stetten is patterned after the Board of Scientific Counselors but on a smaller scale; the Board of Scientific Counselors' meetings last two days, while Dr. Stetten's visits are one day only.

The annual program review identified several areas where new program needs were great. New programs being started this year were chosen: Behavioral Toxicology and Epidemiology. Present programs to receive some increased emphasis are in Mutagenesis (biochemical, mammalian and population genetics, and *in vitro* mutagenesis testing), Pharmacology (marine biomedicine and pharmacology, lung toxicology), Toxicology (Environmental Teratology Information Center), and Biometry (statistical support services). Changes in research emphasis were suggested for certain programs in Toxicology (marsupial program, teratology), Pharmacology (pharmacokinetics, toxication-detoxication), Biophysics (dose-response extrapolations, microwaves), and Mutagenesis (non-mammalian genetics).

The annual review of personnel also evaluates programs, as well as the people who are involved in them. From such reviews, additional information on programs with strength and needs for resources are identified.

The Scientific Director also is working with the Personnel Office and some outside consultants to develop a program for more effective training of scientific managers; for improving communication between scientists, their co-workers, and supporting staffs; and for team building. A Core Group of predoctoral scientist representatives meets regularly with the Scientific Director and Personnel Office people to develop training and job advancement programs for the GS-9 and below predoctoral scientific staff and support people.

At present, the Scientific Director is also involved in recruiting two new branch or laboratory chiefs--one for Environmental Pharmacology, the other for Environmental Biophysics. New program directors for Behavioral Toxicology and Lung Toxicology are also being recruited. This recruiting activity constitutes an appreciable part of the responsibilities of the Scientific Director now and in the foreseeable future.

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH

ENVIRONMENTAL BIOLOGY AND CHEMISTRY BRANCH
Summary Statement

The Environmental Biology and Chemistry Branch focuses on three objectives: to maintain collaborative support laboratories in the disciplines of Chemistry, Inhalation Toxicology, Comparative Pathology, and Veterinary Medicine; to program and coordinate environmental animal procurement, housing, and utilization within the Institute; and to plan and conduct research appropriate to the capabilities of Branch laboratories.

Administratively, the Environmental Biology and Chemistry Branch is organized into four sections: Chemistry, Comparative Biology, Inhalation Toxicology, and Animal Husbandry. Operationally, specific work units (size and composition of each being determined by task requirements) often are drawn from several sections of the Branch.

Support Activities

The Animal Husbandry Section continued to develop and refine competence in a variety of technical animal procedures. The Section routinely provides capabilities in the areas of test diet preparation and feeding, animal breeding (including time-mating and maintenance of inbred stocks), as well as technical services such as administration of test chemicals, forced breeding, and necropsy assistance.

The allocation of space and technical support is now guided by the Institute's Animal Experimentation Committee. The development of such a procedure was necessitated by the demands for animal space exceeding resources.

The committee developed a policy of restricting experimental animal use to specific species, strains, and sources to be used in intramural research. This policy results in a more efficient utilization of space and personnel because several underutilized colonies have been deleted; disease monitoring efforts are focused on fewer stocks. The consolidation of animal sources enabled the scheduling of air charter deliveries to NIEHS. Controlled deliveries under excellent conditions have markedly increased the health status of animals at time of receipt.

A major effort required of the Branch personnel was the development of detailed plans and specifications for the research animal quarters and support laboratories within the permanent NIEHS site.

Since environmental chemistry bridges the gap between environmental analysis and environmental toxicology, chemists are frequently consulted within NIEHS in order to advise scientists in appropriate areas of their research. The support activities of the Chemistry Section fluctuate dependent upon the resources required to meet a particular need. The number of chemical service requests progressively increase, and generally fall into one of two categories: short-term and to the point requests such as compound purity determinations and organic synthesis by published procedures, and a variety of longer term requests which generally require new and improved methodology development. In the latter case, it is difficult to determine the amount of time and effort which will be necessary to complete the task, and a trial period may be necessary to demonstrate feasibility within the limitations of the group. Therefore, for obvious reasons, these latter requests are carefully evaluated and, in general, we seek to do this type of work on a collaborative research support basis.

Proposed guidelines for requesting chemical services have been recently set forth in a chemical services request form which is reviewed by the chemistry section office and then passed on to one of three work groups best suited to handle the request. These work groups also form the basis for research activities within the section. The three work groups are: analytical biochemistry, bioorganic chemistry and synthesis, and specialty instrumentation.

The largest number of short-term requests was handled by the specialty instrumentation group with close to 400 samples analyzed primarily by gas chromatography/mass spectrometric (GC/MS) techniques and to a lesser extent nuclear magnetic resonance (NMR) spectroscopy and high pressure liquid chromatography (HPLC). The samples run included a variety of chemical standards, actual metabolites, and other trace residues which occur in a milieu providing interference.

Requests handled by the analytical biochemistry group required a number of specific approaches. These ranged from specific analyses for various enzymes, vitamins, metabolites, fatty acid esters and urinary calculae to determinations of purity and radiopurity of unlabeled and radioactive labeled chlorinated hydrocarbons.

The more difficult syntheses handled by the bioorganic group included 8(N-fluroenylacetamido)-2'-deoxyguanosine and specifically (^{3}H and ^{14}C) labeled prostaglandin derivatives requiring high chemical purity and radiopurity and unique and known stereochemistry.

As a result of the many diverse requests, research methodology is continually being developed in analytical biochemistry and metabolism, organic chemistry and synthesis, and special instrumental analytical chemistry. A few examples of this were the development of methods for determining diethylstilbestrol (DES) metabolites from mouse liver, gas chromatographic methods for polychlorinated biphenyls (PCB's) on 12 liquid phases of differing selectivity, method for direct analysis of chloroform ($CHCl_3$) in large numbers of drinking water samples, and methods to detect and isolate possible carcinogenic impurities in dimethylhydrazine (DMH) dihydrochloride.

Research Activities

Specific research projects within the Comparative Biology Section interrelate to comprise a program that focuses on the effect of environmental agents on host resistance. This program attempts to define test methods most appropriate for detecting subtle toxicologic effects at levels of exposure below doses associated with the more classical signs of intoxication. Specific effects on the developing immune system were studied using 2,3,7,8-tetrachlorodibenzopdioxin and methyl mercury. Studies on adult resistance to bacterial infection or depressed cellular or humoral immune capabilities were also evaluated using TCDD and selected polychlorinated biphenyl isomers.

Extensive investigations into the pattern and nature of chlorinated dibenzodioxin toxicity are nearing completion. Studies with fourteen specific dioxin isomers in mice and guinea pigs established that toxicity depends upon the number of chlorines present and the specific location of the chlorine atoms. The results show that toxicity between isomers with the same degree of chlorination may differ by orders of magnitude. It was also determined that the nature of the toxicopathologic response in a given species was similar for all dibenzodioxin isomers tested. Thymic and splenic atrophy, toxic hepatitis, vascular lesions (edema and hemorrhage) and serum protein dyscrasias were the prominent features in mice. In contrast, thymic, testicular, and bone marrow atrophy along with adrenal cortical hemorrhage and proliferation of the renal pelvic epithelium were more characteristically found in guinea pigs.

Limited studies with 2,3,7,8 TCDD were initiated using the cynomalgus monkey. These primate studies are to characterize the toxicopathologic effects in a higher species since the pattern of toxic effect varies in lower species. Subsequent primate studies are planned which focus on distribution, storage, and excretion and include enzyme inductive effects.

Comparative studies of the toxicopathologic effects of chlorinated dibenzofurans, contaminants of polychlorinated biphenyls were continued. Early results show the toxicity of this compound to be similar to that for the corresponding dibenzodioxin in the guinea pig. Paradoxically, it is clear that dibenzofuran toxicity is diminished in the rat and mouse. Further studies are in progress, including a primate study. Once toxic effects are produced, the toxicopathologic effects mimic that observed with the chlorinated dibenzodioxins.

Studies were initiated toward determining the dietary factors which accounted for the production of hydronephrosis and hydroureter in rats fed a marginally zinc deficient defined diet. Interim results show that rats fed diets in which the sole sugar source is sucrose develop a high incidence of urinary tract lesions. There was a sex difference in these rats in that the incidence of urinary tract pathology in males was 73.8% while the incidence was only 26.3% in females. The zinc levels employed appeared to have no effect on the incidence of urinary tract pathology.

The Institute program which seeks to establish what health effects, if any, are associated with oral ingestion of asbestos is managed within the Branch. Life-time feeding studies using rats and hamsters that are to be fed one of five specific asbestos types or tremolite in the diet are being done by contract. The program consists of evaluating the possible interactive effects between an asbestos containing diet and a chemical carcinogen known to produce tumors of the intestinal tract. Dose ranging studies with two such carcinogens, methylazoxymethanol and dimethylhydrazine, are in progress at NIEHS. In addition, considerable effort has gone into the development of data handling systems as well as in selecting, procuring, and characterizing the specific asbestos materials to be used.

The Inhalation Toxicology Section conducts several projects aimed at understanding the physiological and biochemical mechanisms whereby airborne agents affect the body, particularly the cardiopulmonary system.

For several years, a number of studies have focused on the toxicity of solvents such as p-xylene and vinyl bromide and whether toxicity of these compounds is altered by pretreatment with known hepatic enzyme stimulators and inhibitors.

In the past year, major effort was concentrated on p-xylene, working out the biochemical basis for cytochrome P-450 inactivation in lung by exposure to p-xylene. Paraxylene was found to be metabolized primarily by the liver through the alcohol and the aldehyde to toluic acid. The liver has sufficient alcohol and aldehyde dehydrogenases to metabolize the xylene to toluic acid. However, during these reactions, some tolualdehyde escapes into the blood stream and returns to the lung. Here, where there is no aldehyde dehydrogenase, the tolualdehyde is metabolized to an as yet unidentified product which inactivates the cytochrome P-450. We have also studied the toxicity of vinyl bromice (VBr) showing that phenobarbital enhances VBr toxicity. We are presently investigating the biochemical mechanisms of VBr toxicity. Recent experiments are exploring the use of oxygen consumption by lung tissue slices as an index of toxicity. Studies are in progress to standardize techniques and determine the effects of cofactors and exogenous substrates on oxygen consumption. The effects of specific pulmonary toxicants, such as bromobenzene and paraquat will be studied using this model. *In vivo* and *in vitro* exposures will be compared.

Efforts to define commercial aerosol product toxicity continued through evaluating two commercial spray starches. Like oven cleaners, acute exposure to spray starches show no gross indication of toxicity after an acute exposure.

The inhalation toxicology contract, whereby Becton Dickenson operates our chronic exposure facility, is in its second years. The contract is intended to identify chronic effects of mixtures of inhaled materials using classical toxicological techniques (organ and body weight, mortality and histopathology). One study attempts to determine the chronic effects of H_2SO_4 mist (10 mg/m^3) and ozone (0.5 ppm) and the combination of the two with two species of animals, rats, and guinea pigs. Results to date

show no synergistic responses. We are also conducting a dose response study using aluminum chloride-hydroxide at three different dose levels (20, 2, and 0.2 mg/m^3), using rats and guinea pigs.

The nature of the research in the Chemistry Section is to some degree predetermined by its support committment and size and space limitations. For example, the chemistry section attempts to provide broad areas of science competence rather than indepth and narrow science expertise. However, to do this requires the availability of diverse instrumentation which consumes space and requires routine maintenance. There are five major research involvements which use selected classes of chemical compounds as models to develop general methodology of value to environmental health scientists:

(1) The first is seeking to develop immunoassay methods which will offer extreme specificity and sensitivity to the detection of hazardous environmental agents and their residues themselves as well as their biological insults such as mutated proteins, etc. at biologically meaningful levels.

Attempts have been made to prepare defined products on coupling haptens to various proteins. Techniques are being developed for the characterization of the conjugates. Relative effectiveness and desirability of various derivatives as antigenic determinants will be assessed.

Chlorinated diphenyl ether derivatives (dibenzodioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants. General active esters for use in coupling to protein were prepared. The stability of the unit linking the hapten nucleus with the activated ester portion was found to be a function of the number of carbon atoms joining the two centers.

Several peculiarities are associated with ether substitution ortho to an aromatic amino group which limit feasible coupling procedures. The Mannich reaction appears to produce only insoluble conjugates. Azo proteins, thus far, have been quite acceptable derivatives.

(2) A second major effort has studied the toxicopathologic and pharmacologic effects of symmetrical hexachlorobiphenyls (HCBs) in chickens and mice taking advantage of available data on their physical-chemical properties. The HCBs represent model PCBs with high and constant chlorine content permitting unequivocal study of a given substitution pattern. Separate and distinct differences in isomer toxicity were observed. A chlorinated dibenzofuran could be differentiated from any of the HCBs studied (in chicks). The major toxicologic and pharmacologic effects observed for the isomers were common to both species tested. Isomer differences in biological response were related to chemical structure.

(3) A third area focuses on the capabilities of various tissue lipases and esterases as early mediators in the interaction of mammals with xenobiotics containing ester linkages. It is of particular interest to understand how lipases are able to bind to and react with water insoluble (lipophilic) compounds such as the herbicides, 2,4-D and 2,4,5-T, phthalate plasticizers, carbamate insecticides, etc. and also if this hydrolytic process is always detoxifying. Rat lung, skin, and pancreas homogenates show distinctive patterns of hydrolytic activity. The major lipase catalyzing hydrolysis has been isolated from rat pancreas and characterized chemically and mechanistically. Hydrolysis of esters of primary and secondary alcohols and phenols can be effected, by what is analogous to a Bac 2 mechanism. Kinetic and isotope incorporation experiments have led to the proposal of a detailed reaction mechanism.

(4) A fourth major involvement has been in the development of special instrumental analytical methodology for application to biological problems. Currently, being emphasized are gas chromatography-mass spectrometric (GC-MS), fouier transform nuclear magnetic resonance (FT-NMR) spectroscopy, and to a lesser extent high pressure liquid chromatography (HPLC).

Modification of our existing high pressure mass spectrometer is nearing completion which will permit the formation and observation of either positive or negative ion beams. It is expected that in the negative ion mode a considerable enhancement in sensitivity to halogenated hydrocarbons will be realized, thereby, facilating their trace level detection in biological and environmental samples. Other work with chemical ionization mass spectrometry (CIMS) in the positive ion mode has demonstrated the suitability of biacetyl as a reagent gas for selectively ionizing compounds with non-bonding electrons or unsaturation and may also be useful for stereoisomer differentiation.

The FT-NMR system is a computerized system with capabilities for observing a number of important nuclei (including ^{1}H, ^{13}C, ^{31}P, ^{15}N and ^{19}F) in a variety of compound classes. The research potential of this instrument, especially in the ^{13}C mode of operation, has barely been explored since we do not have a full time NMR spectroscopist in the group. Several ^{13}C-enriched compounds are available (or soon will be) for research purposes including some specifically labeled 3,4-benzo[a]pyrene oxygenated derivatives and uniformly labeled chlorinated benzenes.

Preliminary studies with diazonium conjugates of amino acids have indicated that ^{13}C-NMR may be useful in the qualitative analysis of haptens and antigens in immunochemistry method development.

HPLC has shown potential for separating closely related isomers and homologs within various classes of halogenated aromatic compounds. The research potential of this instrument will continue to be investigated as, for example, in the preparative mode of operation and for determining partition coefficients.

(5) A fifth area seeks to establish the biomimetric ability of certain well defined (mechanistically) chemical dechlorinating reactions. The ultimate fate of many chlorinated hydrocarbons may be a function of dechlorination propensity. Dechlorination can occur environmentally under a variety of conditions. Dechlorination in biological systems may be both toxifying and detoxifying depending upon the nature of the intermediary and terminal metabolites. Processes known to proceed by way of free radical intermediates have been applied to chlorinated pesticides with the production of dechlorinated and otherwise transformed pesticide derivatives. In addition to making available heretofore rare derivatives of these materials in a regiospecific and stereoselective manner, the mechanisms of dechlorination and isomerization have been revealed in some instances.

Preliminary findings have indicated that the dechlorination of dieldrin in an in vitro system (rat liver tissue) may require the cofactor NADPH and anaerobic conditions.

Other Activities

The section office continues to evaluate the cost versus procedure and benefit relationships for various custom synthesized chemicals (labeled and unlabeled) obtained on contracts. Both technical and administrative problems have delayed our attempts to secure a contractor to guarantee a specified water quality for laboratory animal drinking water.

Nominations to standing committees are accepted without rancor and are usually followed by active participation. Dr. Albro continues to spend a considerable amount (approximately .25 man year) of his time as Chairman of the Institute Editorial Committee. This involvement could impair his ability to perform as project coordinator for the immunochemistry program.

The safety program within the section continues to be expanded. Steps have been taken to monitor laboratory air in order to better relate exposure to possible health problems. In addition, a special hazardous chemical containment facility for the section is in the design stages. The section office cooperates with the safety office in design of specific chemical disposal procedures as needed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30001-05 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Health Surveillance of Laboratory Animals at NIEHS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	EBCB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	J. E. Thigpen	Senior Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

Animal Husbandry Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.2	2.3

SUMMARY OF WORK (200 words or less - underline keywords)

Incoming and resident animals at NIEHS are monitored for spontaneous infections, diseases and parasitism.

The incidence of respiratory disease is very low among laboratory rodents. Enteric pathogens have not been detected. Pinworm infections are moderately frequent but sporadic. No pathologic lesions can be attributed to these nematodes. Hymenolepis nana was detected in three groups of mice.

The incidence of pasteurellosis and mucoid enteritis in NZW rabbits has been greatly reduced since purchasing them from a new source. Infectious disease in guinea pigs has not been encountered. A single batch of feed deficient in vitamin C led to scurvy in experimental guinea pigs. The experiment had to be terminated.

PROJECT DESCRIPTION

OBJECTIVES: To monitor incoming and resident laboratory animals for spontaneous infectious diseases and parasitism.

METHODS EMPLOYED: All research animals at NIEHS are routinely observed for clinical signs of disease. Animals to be selected for health surveillance are chosen one week in advance at a weekly conference. Any problems relating to animal disease or husbandry may be discussed at this meeting.

New species or strains of animals from new sources undergo complete monitoring, including examination for respiratory and enteric pathogens, ecto- and endoparasites and incidental pathology. Reference histological slides are kept on each strain or species. Pooled sera are to be periodically screened for viral serology.

Resident animals are monitored approximately every 6 weeks for respiratory pathogen and parasites. Only tissues from major organs are collected in these cases.

Moribund or dead animals on experiment are referred to the investigator to determine if the condition is spontaneous or experimentally induced. Dead, diseased or abnormal animals are usually submitted to necropsy, after clinical examination.

MAJOR FINDINGS: Incoming and resident rats monitored at monthly intervals have been free of salmonellosis. Chronic respiratory disease (CRD) due to Mycoplasma pulmonis was detected within a single animal room but the incidence of overt clinical disease has been low - usually manifested by labyrinthitis. Occasionally, newly received weanling rats revealed focal corneal opacity and periorbital edema. This condition has proved to be resolving stages of sialodacryoadenitis, a transient viral infection. Lesions usually disappear completely within a week.

Swiss and inbred mice have been relatively free of respiratory infections. Low titers to Sendai virus are encountered but lesions attributable to this respiratory virus have not been seen. Pinworm infections are sporadically encountered. Clinical cases of diarrhea or prolapsed rectum have not been seen. Murine pinworm seems to be inocuous and species related. The presence of this parasite is esthetically displeasing but eliminating the infection is extremely difficult to accomplish. Hymenolepis nana was found in 3 groups of mice on experiment. Affected animals were culled or isolated and measures are being directed toward complete elimination of this parasite. Hamsters have been relatively disease free except for sporadic pinworm infection. Guinea pigs purchased from a reliable SPF colony have been free of infectious disease. A number of animals, well into on experiment, died over a short period of time due to scurvy (Vitamin C deficiency). Investigations revealed a milling error prior to purchase of the feed by NIEHS. Unfortunately, the deficiency was not detected until a large number of animals were

affected. Feed is now routinely analyzed for vitamin C prior to recept by NIEHS.

The incidence of "snuffles" (pasteurellosis) in NZW rabbits has declined considerably since changing source of supply. Mucoid enterites is now rarely encountered among NZW rabbits.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The health status of all research animals must be continually monitored; otherwise, intercurrent infections could cause misinterpretation and possible negation of valuable experimental data. In addition, microbiological and histopathological surveillance has uncovered interesting and valuable data hertofore unpublished in laboratory animal medicine.

PROPOSED COURSE: Surveillance of laboratory animals will continue on a routine basis.

PUBLICATIONS

Gupta, B.N.: Duplication of the Gall Bladder in a Rabbit. Lab. Anim. Sci. 25: 646, 1975.

Gupta, B.N. and Dennis, R.: Tissue Washing Tank for Histologic Preparations. Am. J. Vet. Res. 36: 1403, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30003-04 EBCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of Analytical Methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	EBCB NIEHS
OTHER:	J. R. Hass	Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

As necessary

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

This general project area has as its objective, to develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general, compounds covered by this study will include (1) those for which there is no analytical methodology, insufficiently specific or sensitive methodology, or methodology we are not equipped to utilize, (2) those which are metabolites of compounds for which adequate analytical methodology is available, and (3) those for which an adequate analytical procedure is available but which occur in a milieu providing interference with the standard procedure.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To develop and refine methodology for the quantitative and qualitative determination of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general, compounds covered by this study will include (1) those for which no analytical methodology, insufficiently specific or sensitive methodology, or no methodology we are equipped to utilize are presently available; (2) those which are metabolites of compounds for which adequate analytical methodology is available, and (3) those for which an adequate analytical procedure is available but which occur in a milieu providing interference with the standard procedure.

METHODS EMPLOYED: The analytic methods will generally be developed using gas chromatography, thin-layer and column chromatography, fluorometry, spectrophotometry (IR, UV, Visible), mass spectrometry and isotopic methods. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS: In the present year, methods have been developed for determination of metabolites of diethylstilbestrol from mouse liver, and GLC analysis of polychlorinated biphenyls on 12 liquid phases of differing selectivity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Successful development of the analytical methodology in the areas delineated above will accelerate the successful elaboration of a number of metabolic and degradation studies in progress in the Chemistry Section as well as be of utility for other studies within the Institute.

PROPOSED COURSE: Methodologies will continue to be developed as needed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30005-07 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Toxicity of Dibenzodioxins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
OTHER:	E. E. McConnell	Commissioned Officer	EBCB NIEHS
	M. W. Harris	Biological Laboratory Technician	EBCB NIEHS

COOPERATING UNITS (if any)

K. Chae, Ph.D., Chemistry Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.6	1.3

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to evaluate various dibenzodioxins with regard to the following parameters (1) dose response; (2) lethal dose; (3) clinical signs; (4) clinical pathology; and (5) anatomic pathology in guinea pigs and mice.

PROJECT DESCRIPTION

OBJECTIVES: Using pure chlorinated dibenzodioxins, determine the dose required to produce a toxic or lethal response in laboratory animals. Compare the LD_{50} and lesions produced by the various isomers within an animal species and between species i.e. mice and guinea pigs. Investigate if interactions occur in vivo with the dioxin isomers i.e. are there additive, antagonistic or synergistic effects on toxicity.

METHODS EMPLOYED: Mice, and guinea pigs were given single or multiple doses of various dibenzodioxins (see below) orally in order to study the toxicologic, pathologic and clinical pathologic effects of the intoxication. Standard techniques were used in order to evaluate these effects.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), 1-NH_2-2,3,7,8-TCDD, 1-NO_2-2,3,7,8 TCDD, 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (HCDD), 1,2,3,6,7,8-HCDD, 1,2,3,7,8,9-HCDD, 2,8-dichlorodibenzo-p-dioxin, 2,3,7-trichlorodibenzo-p-dioxin (Tri CDD), 1-NH_2-3,7,8-Tri CDD, 1-NO_2-3,7,8-Tri CDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PCDD) and 1,2,4,7,8-PCDD.

MAJOR FINDINGS: The toxicopathology of the above compounds was studied in C57Bl/6gh (J67) mice and Hartley guinea pigs. The single oral LD_{50} was determined for these compounds in both species of animals (Table). 2,3,7,8-TCDD was found more toxic than any of the other isomers. However, 1,2,3,7,8-PCDD was almost as toxic. The lesions produced by all compounds were similar in a given species. Thymic and splenic atrophy, toxic hepatitis, vascular lesions (edema and hemorrhage) and serum protein dyscrasias were the prominent features in mice. In contrast, thymic, testicular and bone marrow atrophy along with adrenal cortical hemorrhage and proliferation of the renal pelvic epithelium were more characteristically found in guinea pigs.

ESTIMATED ORAL LD_{50-30}[a] OF SELECTED CHLORINATED DIBENZO-P-DIOXINS

Chlorination	Guinea pigs		Mice	
	µg/Kg	µmole/Kg	µg/Kg	µmole/Kg
2,8	300,000	1,180	-	-
2,3,7	20,439	71.0	3,000	10
2,3,7,8	2	0.006	283.7	0.88
1,2,3,7,8	3.1	0.009	337.5	0.94
1,2,4,7,8	1,125	3.15	5,000	14
1,2,3,4,7,8	72.5	0.185	825.0	2.11
1,2,3,6,7,8	70 - 100	0.179-0.255	1,250	3.19
1,2,3,7,8,9	60 - 100	0.153-0.255	-	-
1-NO_2-3,7,8	30,000	90.0	-	-
1,NH_2-3,7,8	30,000	99.0	-	-
1-NO_2-2,3,7,8	47.5	0.129	2,000	5.4
1-NH_2-2,3,7,8	194.2	0.576	4,800	14.2

[a] Spearman-Karber Method

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzodioxins may occur in association with many useful chlorinated cyclic compounds. The symmetrical tetrachloro isomer is one of the most toxic chemicals known. The pattern of toxicity associated with most of the isomers has not been determined. The interactions of isomers as they relate to toxicity is unknown. These facts must be determined if assessment of the environmental health implication of products which contain dibenzodioxin isomers as contaminants are to be made.

PROPOSED COURSE: This phase of dioxin investigations is completed.

PUBLICATIONS

Vos, J.G., Moore, J.A. and Zinkl, J.G.: Toxicology of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in C57Bl/6 Mice. Toxicol. Appl. Pharmacol. 29: 229-241, 1974.

McConnell, E.E. and Moore, J.A.: The Comparative Toxicity of Chlorinated Dibenzo-p-dioxin Isomers in Mice and Guinea Pigs. Toxicol. Appl. Pharmacol. (Abstract) (In Press).

McConnell, E.E., Moore, J.A. and Harris, M.W.: The Comparative Toxicity of Chlorinated Dibenzo-p-dioxin Isomers in Mice and Guinea Pigs. (In preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30007-04 EBCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effect of Environmental Chemicals on Immunocompetence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Faith	Staff Fellow	EBCB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	M. Biocca	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	0.9

SUMMARY OF WORK (200 words or less - underline keywords)

These studies were undertaken to investigate the effects of selected environmental chemicals on the immune competence of laboratory animals. The topics of present interest include (1) effects of transplacental exposure; (2) effects of neonatal exposure; and (3) effects of adult exposure. Chemicals investigated to date are 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3,4,5,3',4',5'-hexachlorobiphenyl (HCB), and methyl mercury. Results of studies completed to date show TCDD to profoundly suppress cell mediated immune reactions, HCB to suppress immune response to bacterial lipopolysacchamide, and methyl mercury not to have significant effects at the time periods tested.

PROJECT DESCRIPTION

OBJECTIVES: To determine the effects, if any, of selected environmental chemicals on the immune competence of laboratory animals and to develop a standard methodology for assessing the effects of environmental chemicals on the immune response.

METHODS EMPLOYED: Cell mediated (T-cell) immunity is being assessed in vitro by the response of thymic or splenic cell cultures to the mitogens Concanavalin A or phytohemagglutinin and in vivo by delayed hypersensitivity responses to the contact sensitizing agent 4-ethoxymethylene-2-phenyl-2-oxazolone (oxazolone) and tuburculin reactions. Humoral immunity (B-cell mediated) is being assessed by the response of splenic cell cultures to the mitogens E. Coli 055 B5 lipopolysacchamide and dextran sulfate (B-cell mitogens) and by passive hemagglutination or radial diffusion to measure antibody responses to both T-independent and T-dependent antigens. In addition to immunologic parameters animals are observed for signs of general toxicity.

MAJOR FINDINGS: Results of studies with TCDD completed to date have confirmed and extended the earlier studies of Vos and Moore. Rats exposed to TCDD prenatally and postnatally have depressed body weights and depressed cellular immune responses. This depression last for as long as the animals have been observed, 145 days (124 days after the last exposure to TCDD). Rats exposed to TCDD postnatally show a depression in body weight through age 59 days. At 145 days of age those animals have normal body size but are still suppressed in their ability to mount cell mediated immune responses. Cell mediated responses are suppressed both as response to mitogens in vitro and delayed hypersensitivity responses. Humoral immune responses of TCDD exposed rats to bovine gamma globulin (BGG) are not suppressed. This indicates that TCDD does not interfere with the normal functioning of all T-cell subsets as BGG is a T-dependent antigen and requires functioning T-helper cells for a normal response.

The studies completed to date with methyl mercury have shown methyl mercury to have no significant effect on immune competence at the intervals studied (2-3 months post exposure).

HCB has been shown to suppress the antibody response of mice to E. coli lipopolysacchamide but not to sheep red blood cells when fed at levels of 1 and 3 parts per million in the feed for 4 weeks.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental contaminants may exert subtle effects on man and animals. Among these effects may be a modulation of immune response, either suppression or enhancement. It is important to establish which environmental chemicals may induce modulation of immune responses, and their mode of action, in order to evaluate the hazards of immune modulation for man and animals.

PROPOSED COURSE: To be continued.

PUBLICATIONS

Moore, J.A., and Faith, R.E.: Immunologic Response and Factors Affecting Its Assessment. Environmental Health Perspectives (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30008-04 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Effects of Environmental Chemicals on Host Resistance to Infectious Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Thigpen	Senior Staff Fellow	EBCB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	E. E. McConnell	Commissioned Officer	EBCB NIEHS
	R. E. Faith	Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)
Joe Haseman, Ph.D., Environmental Biometry Branch
Histology Section, EBCB
Animal Housing

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.55	0.20	0.35

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of subclinical levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the response of mice to infection with either Salmonella bern or Herpesvirus suis, also known as pseudorabies virus, are reported. Sublethal levels of TCDD were used: 0.5, 1, 5, 10 or 20 µg/kg, given through a gastric tube once weekly for 4 weeks. A significant decrease in weight gain compared with control mice occurred at the 20-µg dosage. Dose schedules of 1 µg or more, followed by salmonella infection, resulted in significant increases in mortality and decreases in the time from infection to death. However, TCDD had no significant effect on mortality in the pseudorabies-infected mice. The most important finding in this study is that extremely low levels of TCDD, which do not produce clinical or pathological change, still have the capacity to affect host defense.

PROJECT DESCRIPTION

OBJECTIVES: To develop test systems which determine the effects of environmental pollutants on disease resistance of laboratory animals as measured by mortality rates subsequent to exposure to infectious agents. More specifically to determine whether any interaction (antagonistic or synergistic) occurs in dioxin (TCDD) exposed mice when infected with a bacterium (Salmonella bern) or a virus (Pseudorabies-PRV).

METHODS EMPLOYED: Pseudorabies virus was produced in quantity employing rabbit kidney (RK-13) monolayers and stored in aliquots at -70°C until ready for use. A lethal dose response curve was established in 8 week-old mice, by inoculating 20 mice/group with 0.2 ml (IP) of varying dilutions of PRV. A LD_{20} dose was determined and used as the viral inoculum in the blind study.

Salmonella bern was grown overnight on blood agar and a bacterial suspension prepared in 0.85% saline. Bacterial concentrations were determined by the spectrophotometer (% transmittance - 540 mμ) and confirmed by total plate counts (TPC). A lethal dose response curve was established in C57Bl/6JFH (J67) normal mice by inoculating 0.2 ml (IP) of various dilutions of bacteria. Mortality rates were determined for 14 days with the bacteria and virus inoculated mice and the dead mice examined by both histologic and microbiologic methods to establish the cause of death.

Experimental Design (Blind-Study): Experiment #1 - 3 treatment groups (5, 10, and 20 μg/Kg body wt.) and control group (60 mice/group randomly distributed) of 5-week-old mice were dosed each week by gastric intubation for 4 weeks with the appropriate chemical (TCDD and Carrier) and carrier alone (controls). Two days after last treatment (8 weeks-old), the 4 groups of 60 mice/group were randomly split into 3 groups of 20 mice/group and inoculated with the LD_{20} dose of Salmonella bern, PRV or saline (diluent). Mortality rates were recorded daily for 14 days in all groups. Mortality rates in the 3 TCDD exposed groups were compared to mortality rates in the control. A comparison was also made between bacteria and virus groups. All data was analyzed statistically by Dr. Haseman. Three mice from each group was examined by both histologic and microbiologic methods to establish cause of death.

Experiment #2 was conducted identically to the first except that each mouse received either 0, 0.5, 1.0 or 5.0 μg of TCDD/Kg per week and the PRV subgroup was eliminated.

MAJOR FINDINGS: Effects of TCDD on weight gain: There was essentially no difference in mean group body weights prior to TCDD dosing. By week 4, the 20 μg of TCDD/Kg gained significantly ($P<0.01$) less weight than did the controls. The 10 μg of TCDD/Kg dose group also gained less weight; all other TCDD groups were comparable.

Effects of TCDD on mortality rates: The groups of mice exposed to 1, 5, 10, or 20 μg of TCDD/Kg and given S. bern showed a significant ($P<0.05$ to 0.01) increase in mortality rates. The mortality rate in the 0.5 μg of TCDD/Kg dose group was identical to the control group; therefore, the "no-effect" level based on the parameters of this study would be somewhere between 0.5 and 1.0 μg of TCDD/Kg.

The mortality rates in the groups that received virus did not differ significantly with variations in the dose of TCDD.

Effects of TCDD on Time from Infection to Death: There was a significant ($P<0.01$) dose-response decrease in time from infection to death in the S. bern challenged mice.

The most important finding in this study is that extremely low levels of TCDD, which do not produce clinical or pathological change, still have the capacity to affect host defense.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A variety of environmental chemicals when administered in sublethal doses are immunosuppressive and increased mortality rates have been observed upon exposure to infectious agents. These findings are significant in that humans may be exposed to these same compounds in minute quantities and, therefore, may be rendered more susceptible to certain types of infectious agents. This bacterial and viral test system is highly reproducible and thus we can (1) test other environmental chemicals on a comparative basis utilizing this procedure; (2) the test system can be employed in co-operative studies (with other NIEHS scientists) in which one wishes to determine the effects of microwave, noise pollution, or aerosol exposure to toxic agents, etc. on the host resistance to bacterial or viral infections.

PROPOSED COURSE: To be continued - Mechanisms of decreased host resistance as a result of TCDD exposure. (1) To elucidate the role of the thymus (T-cells) in host resistance as determined by mortality rates in mice (C-57) exposed to TCDD and subsequently infected with a LD_{20} dose of Pneumococcus. T-cells do not play a significant role in host defense against Pneumococcus infection in nude mice. (Ref: Inf. & Imm. 12:1222-1223, 1975). (2) Plans are underway to investigate the functional capabilities (both in vitro and in vivo) of phagocytic cells from TCDD exposed mice.

PUBLICATIONS

Thigpen, J.E., Faith, R.E., McConnell, E.E. and Moore, J.A.: Increased Susceptibility to Bacterial Infection as a Sequela of Exposure to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. Infection and Immunity 12(6): 1319-1324, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30010-02 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunoassay Techniques for Detection and Quantitation of Environmental Chemicals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Faith	Staff Fellow	EBCB NIEHS
OTHER:	P. W. Albro	Research Chemist	EBCB NIEHS
	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

These studies were undertaken to develop methodology for immunoassay of environmental chemicals. Current interest centers around chemicals which are lipid soluble in contrast to those which are water soluble. Chemicals currently under investigation are 2,3,7,8-tetrachlorodibenzo-p-dioxin and a model chlorinated biphenyl. Areas of present interest include (1) methods for coupling of haptenic environmental chemicals to carrier molecules to be be used as antigens; (2) effects of halogens on immune response; and (3) methods for immunoassay of environmental chemicals.

PROJECT DESCRIPTION

OBJECTIVES: To develop methodology for immunoassay of environmental chemicals.

METHODS EMPLOYED: Presently rabbits are being immunized with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) - protein conjugates or the model chlorinated biphenyl - protein conjugates emulsified in complete Freund's adjuvant. Antibody production is being monitored by means of double diffusion in gel. Antibodies obtained from these immunizations will be utilized to develop methodology for immunoassay of environmental chemicals.

MAJOR FINDINGS: The initial group of rabbits placed in this study are just beginning to make antibody to TCDD after being immunized repeatedly over a period of time approximately a year in length. Initial efforts are being made towards developing immunoassay methodology for TCDD utilizing these antibodies.

A study is being undertaken to investigate the effects of halogens on the immune response to haptenic compounds. Halogenated analogs of the well defined haptens dinitrophenyl and dinitroanaline are coupled to carrier proteins and injected into mice. The response of these mice will be monitored and the antigenicity of the various compounds compared.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Specific, highly sensitive assays for TCDD and other environmental chemicals would be very useful in studies of environmental contamination, body burden and distribution, etc. with these chemicals. This study will hopefully develop the methodology required for specific and sensitive assays for environmental chemicals.

PROPOSED COURSE: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30012-03 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Comparative Chemistry and Biological Properties of Selected Hexachlorobiphenyl Isomers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
OTHER:	K. Chae	Staff Fellow	EBCB NIEHS
	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	M. Biocca	Visiting Fellow	EBCB NIEHS
	L. A. Levy	Research Chemist	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	J. Goldstein	Research Pharmacologist	NERC EPA
	J. R. Hass	Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

Comparative Biology Section, EBCB, and Pesticides and Toxic Substances Lab, NERC, EPA, Research Triangle Park, North Carolina

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.9	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The toxicopathologic and pharmacologic effects of symmetrical hexachlorobiphenyls (HCBs) were studied in chickens and mice. The HCBs represent model PCBs with high and constant chlorine content permitting unequivocal study of a given stustitution pattern. Separate and distinct differences in isomer toxicity were observed, and one could differentiate a chlorinated dibenzofuran effect from any HCB studied (in chicks). Although certain toxicologic and pharmacologic characteristics differentiate the biological responses in each species tested, the major effects were common to both chicks and mice. These isomer differences in biological response can be related to chemical structure.

PROJECT DESCRIPTION

OBJECTIVES: (1) To begin to clarify and define the biological effects of commercial PCB-mixtures by systematically examining the chemistry and biological effects of individual selected PCBs from synthesis. (2) To correlate the physical-chemical properties of selected symmetrical hexachlorobiphenyls (HCBs) with toxicity. The HCBs with high chlorine content should represent an environmentally persistent class of PCBs.

METHODS EMPLOYED: The chemistry of the HCB isomers was defined using a variety of synthetic, chromatographic, and spectroscopic techniques. Such biological responses as mortality, body weight change, food consumption, body organ weights, gross and microscopic pathology, incidence of edema and porphyria, liver enzyme induction, etc. were measured simultaneously. Chromatographic and spectroscopic techniques were also used to isolate and identify metabolites. Several of the desired HCB isomers were obtained through total synthesis in our laboratories.

MAJOR FINDINGS: The symmetrical hexachlorobiphenyls (HCBs) represent model PCBs with high and constant chlorine content permitting unequivocal study of a given substitution pattern. Separate and distinct differences in isomer toxicity to chicks and mice were observed comparing such parameters as mortality, body weight gain, liver effects, porphyrin accumulation, involution of the lymphatic organs, fluid accumulation, and pathologic effects. Although certain toxicologic characteristics differentiate the biological responses in each species tested, the major toxicopathologic effects were common to both chicks and mice. These differences can be related to chemical structure *via* effects of varying chlorine substitution on compound lipophilicity and metabolism. Relative molecular polarizability can be correlated with lipophilicity and biological activity and is measurable by chromatographic and spectroscopic techniques. These parameters were highest in HCBs with planar symmetry and 4,4'-substitution. The rates of metabolism of these isomers are considered of lesser importance than the potentially highly toxic nature of some of the intermediary and terminal metabolites, although overall toxicity may be largely due to varying degrees of lipophilicity. The decreasing order of overall toxicity observed was 3,4,5,3',4',5'-HCB >>2,4,6, 2',4',6'-HCB >2,3,4,2',3',4'-, 2,4,5,2',4',5'- >2,3,6,2',3',6'-HCB.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use and occurrence of the polychlorinated biphenyls has necessitated investigations of their possible human health effects. In order to facilitate such studies, systematic examination of purified isomers is necessary to obtain clarity and definition of the biological effects.

PROPOSED COURSE: (1) In order to complete these studies we plan to investigate the toxicity of the only remaining symmetrical hexachlorobiphenyl isomer (2,3,5,2',3',5') having predicted its toxicity on the basis of its chromatographic and spectroscopic properties. (2) At the

same time, we would like to look at the toxicity of a few selected simple mixtures of these HCBs at various concentrations in the diet. (3) We want to study the metabolism and pharmacokinetics of the 3,4,5, 3',4',5'-, 2,4,6,2',4',6'- and 2,3,6,2',3',6'-HCB isomers in more detail in view of the considerable differences in toxicity and the possibility of dibenzofuran formation.

PUBLICATIONS

McKinney, J.D.: Toxicology of Selected Symmetrical Hexachlorobiphenyls. Correlating Biological Effects with Chemical Structure. Proceedings of National Conference on PCB's, Chicago, Illinois, November, In Press, 1975.

Biocca, M., Moore, J.A., Gupta, B.N. and McKinney, J.D.: Toxicology of Selected Symmetrical Hexachlorobiphenyls. Biological Responses in Chicks and Mice. Proceedings of National Conference on PCB's, Chicago, Illinois, November, In Press, 1975.

McKinney, J.D., Chae, K., Moore, J.A., and Goldstein, J.A.: Toxicological Assessment of Hexachlorobiphenyl Isomers and 2,3,7,8-Tetrachlorodibenzofuran in Chicks. I. Relationship of Chemical Parameters. Toxicol. Appl. Pharmacol., In Press, 1976.

Goldstein, J.A., McKinney, J.D., Lucier, G.W., Hickman, P., Bergman, H. and Moore, J.A.: Toxicological Assessment of Hexachlorobiphenyl Isomers and 2,3,7,8-Tetrachlorodibenzofuran in Chicks. II. Effects on Drug Metabolism and Porphyrin Accumulation. Toxicol. Appl. Pharmacol., In Press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30013-02 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Identification of the Metabolites of Selected Hexachlorobiphenyls in the Chicken

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Hass	Staff Fellow	EBCB NIEHS
OTHER:	K. C. Chae	Staff Fellow	EBCB NIEHS
	J. D. McKinney	Supervisory Research Chemist	

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The symmetric hexachlorobiphenyls (HCB) 2,4,6,2',4',6'-HCB, 2,3,6,2',3',6'-HCB, 2,4,5,2',4',5'-HCB, 2,3,4,2',3',4'-HCB and 3,4,5,3',4',5'-HCB were fed to chicks and the excreta collected and extracted. The metabolites isolated were identified by gas chromatography-mass spectrometry (GCMS). No metabolites were found for the 3,4,5,3',4',5'-HCB or the 2,3,4,2',3',4'-HCB. One hydroxy metabolite was found for the 2,3,6,2',3',6'-HCB but not in sufficient quantity to allow further characterization. The 2,4,6,2',4',6'-HCB was found to be metabolized to a methylpentachlorobiphenyl, a methylhexachlorobiphenyl, 2,4,6,2',3',4'-HCB and a pentachlorobiphenyl. The 2,4,5,2',4',5'-HCB was found to be metabolized to 3 hydroxy 2,4,5,2',4',5'-HCB, a hexachlorobiphenylquinone and a pentachlorobiphenylquinone. The latter two were probably formed from the corresponding dihydroxyl compounds during sample work-up.

PHS-6040

PROJECT DESCRIPTION

OBJECTIVES: To identify metabolites of selected hexachlorobiphenyls (HCBs) isolated from chicken excreta.

METHODS EMPLOYED: Extracts of excreta from chickens which had been fed 2,4,6,2',4',6'-HCB, 2,4,5,2',4',5'-HCB, 2,3,4,2',3',4'-HCB or 3,4,5,3',4',5'-HCB were fractionated by Florsil chromatography. The various fractions were then analyzed by gas chromatography and combined gas chromatography/mass spectrometry. Both low and high resolution mass spectrometry were used.

MAJOR FINDINGS: Both the degree of metabolism and types of metabolites formed depend upon the substitution pattern around the aromatic ring. No metabolites were found for either the 2,3,4,2',3',4'-HCB or the 3,4,5,3',4',5'-HCB. The 2,3,6,2',3',6'-HCB was metabolized to a hydroxy-HCB, but insufficient material was available for complete identification. More extensive formation of oxygenated compounds was found in the case of 2,4,5,2',4',5'-HCB. In particular 3-hydroxy-2,4,5,2',4',5'-HCB, a HCB-quinone and a pentachlorobiphenyl quinone were isolated. The latter two compounds most likely are formed during sample clean-up from the corresponding dihydroxy chlorobiphenyls. Assuming no chlorine scrambling in the parent-HCB, this would indicate the dihydroxy metabolite to be 3,6-dihydroxy-2,4,5,2',4',5'-HCB. The 2,4,6,2',4',6'-HCB was metabolized to a methylpentachlorobiphenyl, a methylhexachlorobiphenyl, 2,4,6,2',4',6'-HCB and a pentachlorobiphenyl. Microbial metabolism can not be ruled out.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is part of a larger study at the Institute to determine the toxicity and metabolic fate of individual chlorinated biphenyl.

PROPOSED COURSE: Complete identification of metabolites from excreta and in tissue and then terminate. The metabolites found in chick excreta for the only remaining symmetrical HCB isomer (2,3,5,2',3',5') will be identified as a part of a proposed study to determine its relative toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30014-02 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Identification of the Metabolites of 4-Chlorobiphenyl and 4,4'-Dichlorobiphenyl in the Rat.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Hass	Staff Fellow	EBCB NIEHS
	H. B. Matthews	Research Chemist	PB NIEHS
	L. T. Jao	Visiting Fellow	PB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

Pharmacology Branch, NIEHS

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The metabolites of 4-chlorobiphenyl (MCB) and 4,4'-dichlorobiphenyl (DCB) were isolated and cleaned-up using thin-layer chromatography. The metabolites were then identified. The MCB metabolites were found to be 3-hydroxy-4'-chlorobiphenyl, 4-hydroxy-4'-chlorobiphenyl, 3,4-dihydroxy-4'-chlorobiphenyl and either 3-hydroxy-4-methoxy-4'-chlorobiphenyl or 3-methoxy-4-hydroxy-4'-chlorobiphenyl. The DCB was metabolized largely to 3-hydroxy-4,4'-dichlorobiphenyl and to a minor amount of 2-hydroxy-4,4-dichlorobiphenyl.

PROJECT DESCRIPTION

OBJECTIVES. To identify the metabolites of 4-chlorobiphenyl and 4,4'-dichlorobiphenyl in the rat.

METHODS EMPLOYED: Rats were fed 30 mg/kg/day of the respective radiolabeled compounds. The urine and feces were extracted and the extracts cleaned up by thin layer chromatography (TLC) using radiography to locate the metabolites. Each TLC spot was then analyzed by means of gas chromatography, combined gas chromatography-mass spectrometry, proton and carbon-13 nuclear magnetic resonance spectroscopy and infrared spectroscopy. The metabolites were identified by comparison of this data with that of standard compounds or by empirical interpretation of the various spectra. The radioactivity measurements for each metabolite was used to calculate its percentage of the recovered radioactivity.

MAJOR FINDINGS: Four metabolites were found for the 4-chlorobiphenyl. These were 3-hydroxy-4'-chlorobiphenyl (70%), 4-hydroxy-4'-chlorobiphenyl (65%), 3,4-dihydroxy-4'-chlorobiphenyl (22%) and either 3-hydroxy-4-methoxy-4'-chlorobiphenyl or 3-methoxy-4-hydroxy-4'-chlorobiphenyl (2.5%). This accounts for 92% of the radioactivity excreted in the urine and 70% in the feces. Two metabolites were found for the 4,4'-dichlorobiphenyl. These were 3-hydroxy-4,4'-dichlorobiphenyl 91% and a trace of 2-hydroxy-4,4'-dichlorobiphenyl. These two compounds accounted for 90% of the excreted radioactivity.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project is part of a larger study at the Institute to determine the toxicity and metabolic fate of individual chlorinated biphenyls.

PROPOSED COURSE: Complete the identification of the methoxy-hydroxy compound and then terminate the project.

PUBLICATIONS

Hass, J.R., Jao, L.T., Matthews, H.B. and Wilson, N.K.: *In Vivo* Metabolism of Radioactive 4-Chlorobiphenyl and 4,4'-Dichlorobiphenyl in Rats. Society of Toxicology, 15th Annual Meeting, Atlanta, GA, March 14-18, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30015-02 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies in Chemical Ionization Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. R. Hass Staff Fellow EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

Department of Chemistry, University of North Carolina, Chapel Hill, NC

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The application of biacetyl as a reagent gas in chemical ionization (CI) mass spectrometry (MS) is being investigated. A preliminary study of several classes of compounds showed that biacetyl protonated all the compounds studied with non-bonding electrons or unsaturation. Unsaturated compounds also undergo the formation of an $(M+43)^+$ species. Esters of cis and trans-4-t-butylcyclohexanol and endo and exo norbornane 2,3-diol were investigated using biacetyl as the reagent gas for CIMS. It was found that by careful control of source conditions, the less sterically blocked ester would undergo acetylation whereas the more sterically crowded ester would not.

PROJECT DESCRIPTION

OBJECTIVES: The aim of this project is to develop qualitative analytical methods applicable to biomedical samples based upon the use of selective reagent gases in chemical ionization (CI) mass spectrometry (MS). It is anticipated that such methods will in turn improve our capabilities in the area of sample identification where insufficient material is available for other methods to be used.

METHODS EMPLOYED: The NIEHS high pressure mass spectrometer is being used in this work. Biacetyl was chosen as the first reagent gas for study in view of the literature available describing its gaseous ion chemistry. Since polar compounds tend to form cluster ions at high pressure, helium has been used to dilute the biacetyl while maintaining the source pressure sufficiently high for CI to occur. Most samples of biomedical interest require the use of gas chromatography in conjunction with mass spectrometry. Therefore, the gas chromatograph was used for sample introduction.

MAJOR FINDINGS: Biacetyl has been found suitable as a reagent gas for CIMS. The background ion current has three major ions at m/e 43, 87, and 129 which do not offer a serious interference with sample ion currents. Furthermore, biacetyl has been found to selectively ionize compounds with non-bonding electrons through proton transfer and unsaturated compounds by proton transfer and acetylation. Aliphatic compounds are not ionized as well as olefinic or aromatic compounds.

A study of stereoisomeric esters is in progress. The results thus far indicate that biacetyl might be useful as a CI reagent gas to gain information about the steric environment of these functional groups. In particular, trans-4-t-butylcyclohexylacetate is significantly more reactive toward acetylation by biacetyl than is the cis isomer. The same selectivity is observed for the proprionate analogues of these compounds as well as for these esters of endo and exo-norboman-2,3-diol.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The application of mass spectrometry to problems in biomedical analysis in recent years has been particularly fruitful. Chemical ionization techniques have provided information complementary to data acquired using electron impact ionization methods. This project should allow a means to gather further information concerning the nature of an unknown sample.

PROPOSED COURSE: The study in progress will be completed. Following that other reagent gases will be evaluated for their regiospecificity and stereoselectivity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30017-03 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biomimetic Model for the Study of Dechlorination Processes of Pesticides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. A. Levy	Research Chemist	EBCB NIEHS
OTHER:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Processes known to proceed by way of free radical intermediates have been applied to chlorinated pesticides with the production of dechlorinated and otherwise transformed pesticide derivatives. In addition to making available heretofore rare derivatives of these materials in a regiospecific and stereoselective manner, the mechanisms of dechlorination and isomerization have been revealed in some instances.

PROJECT DESCRIPTION

OBJECTIVES: To develop dechlorination reactions whose mechanism is understood and which involve defined intermediates. Analysis of the products of the dechlorination of target pesticide molecules and comparison with the results of environmental and/or biological dechlorination will aid in clarifying the mechanisms of environmental degradation and metabolic activation.

METHODS EMPLOYED: Photochemical processes, metal hydride reduction. Preparation of standards by unambiguous synthetic methods. Mass spectroscopy, nmr spectroscopy, infrared spectroscopy. Chromatography (glc, thin layer, column, hplc).

MAJOR FINDINGS: A number of chlorinated pesticides and derivatives obtained from them by environmental action have been dechlorinated using chemical (metal hydride) and physical (ultraviolet irradiation) systems known to involve free radical intermediates. The stereochemistry and regiospecificity of reductive dechlorination as a function of the reducing system as well as the hybridization of the carbon atom being reduced and other more remote structural changes have been determined.

The dichotomy present in reductive photodechlorination vs photorearrangement as a function of the reaction conditions has been resolved. From this work has come an understanding of certain radical processes.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The chlorinated pesticides are recognized to be major environmental concern due to their widespread distribution, their persistance and their concentration in the food chain. They are slowly converted to lesser chlorinated species, about which little is known. Some of these compounds represent radically new structural types. A knowledge of the processes by which these transformations take place would make possible more comprehensive biological and toxicological strides.

PROPOSED COURSE: To continue the study of the correlation of laboratory models of dechlorination with environmental processes. To develop reliable synthetic methods to produce rare and important isomers of pesticides of importance to other ongoing investigations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30018-03 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Ester Hydrolases at the Environmental Interface

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	EBCB NIEHS
OTHER:	J. R. Hass	Staff Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to evaluate the capabilities of various tissue lipases and esterases as early mediators in the interactions of animals with xenobiotics containing ester linkages. The major lipase catalyzing hydrolysis of phthalate esters, herbicide esters (e.g. 2,4-D esters) etc. has been isolated from rat pancreas and characterized chemically and mechanistically.

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the capabilities of various tissue lipases and esterases as early mediators in the interaction of animals with xenobiotics containing ester linkages.

METHODS EMPLOYED: Most studies will involve the rat, but significant findings will be checked in mice, hamsters, guinea pigs and rabbits. Lung, gastrointestinal, and skin enzymes will be studied, using techniques generally described previously (Biochim. Biophys. Acta, 360: 380, 1973). Test substances will initially include: (1) esters of 2,4-D and 2,4,5-T, (2) phthalate plasticizers, (3) detergents such as Tween 20, Tween 80, (4) aromatic rubrefacients such as methyl paraben, menthyl salicylate, (5) volatile solvents such as ethyl acetate, butyl acetate, (6) carbamates.

MAJOR FINDINGS: The nonspecific lipase from rat pancreas catalyzes the hydrolysis of esters of primary and secondary alcohols and phenols, by what is analogous to a Bac 2 mechanism. Kinetic and isotope incorporation experiments have led to the proposal of a detailed reaction mechanism.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Relatively little is known about non-specific lipase activity, since the enzyme(s) has(have) never previously been purified. Although a great deal is known about lung esterases, very little is known about lung lipases (hydrolases for insoluble esters). Virtually nothing is known about ester hydrolases in skin. These enzymes may either protect against some hazardous compounds by converting them to less easily absorbed, more polar materials, or increase the hazard of other compounds when the acid (or alcohol) is more toxic than the ester. A second anticipated result of this project should be a better understanding of how enzymes in general are able to bind to and react with water-insoluble compounds.

PROPOSED COURSE: It is anticipated that one or more of the following studies will be begun shortly, depending upon the availability of personnel: (1) lung lipases, their substrate specificities and general kinetic properties, (2) skin lipases and esterases: do they exist?

PUBLICATIONS

Albro, P.W., Corbett, B.J. and Latimer, A.D.: Purification and Partial Characterization of Nonspecific Lipase from Rat Pancreas. Biochim. Biophys. Acta, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30019-04 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Hydrocarbons as Model Compounds for Metabolic Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: P. W. Albro Research Chemist EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this study are to determine to what extent hydrocarbons may be used as model compounds in predicting modification of aliphatic chains of other compound classes in vivo. Isoprenoid and other simple types of hydrocarbons have been used. Unexpected metabolites, including a tertiary alcohol have been detected.

PROJECT DESCRIPTION

OBJECTIVES: To determine to what extent hydrocarbons may be used as model compounds in predicting metabolic modification of aliphatic chains in other classes of compounds.

METHODS EMPLOYED: The basic test organism is the rat, to which hydrocarbons are administered orally in corn oil, mixed with rat chow, injected I.P., I.V. or I.M. In some cases radioactive tracers (1-^{14}C-hexadecane, 1-^{14}C-octadecane, and U-^{14}C-phytane and 2,3-^{3}H-phytane synthesized here) are used in metabolic studies. Standard chromatographic, immunologic, radioassay and other biochemical techniques are used, except that balance studies of absorption from the gut are made as described in Biochim. Biophys. Acta 219: 437-446, 1970.

MAJOR FINDINGS: No work was done on this project during the past year.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Biological mechanisms for the modification and degradation of aromatic, alicyclic and aliphatic moieties are far from adequately understood. An understanding of in vivo capabilities in these areas will simplify metabolic studies of many classes of compounds, and hopefully permit reliable predictions as to what types of metabolites might be expected upon ingestion of various new drugs, pesticides, food additives, etc.

PROPOSED COURSE: Changing priorities make it necessary to terminate this project.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30020-05 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Transport and Metabolism of Phthalate Esters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	EBCB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

E. E. McConnell, D.V.M., Comparative Biology Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project have been to elaborate various aspects of the transport, absorption, excretion, and metabolism of phthalate esters in the rat. Metabolic fates of both the acid and alcohol moieties have been studied. The enzyme primarilly responsible for hydrolysis of phthalate esters in vivo has been purified and characterized. A modified phthalate diester that is not metabolized in rats has been studied and found to have very low, if any, toxicity.

PROJECT DESCRIPTION

OBJECTIVES: To prepare purified standard labeled and unlabeled phthalate esters and to elaborate aspects of their transport, absorption, metabolism, excretion and tissue residues following various routes of administration in rats.

METHODS EMPLOYED: Chromatography, spectrophotometry, mass spectrometry, isotopic methods, standard enzymology techniques.

MAJOR FINDINGS: 2-Ethylhexan-1-ol (1-^{14}C) was synthesized and used in metabolic studies. The urinary metabolites were identified and a catabolic pathway proposed. Di(ethylhexyl) tetrachlorophthalate was not absorbed from the GI tract of rats, and was not metabolized following IP injection. No pathologic effects were seen at doses up to 1g/Kg (oral or IP) in rats.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The phthalic acid esters are important industrial chemicals employed with extensive utility, primarily as plasticizers. Recent evidence of their migration into human tissues as well as their increasing occurrence in the ecology have been cited. Knowledge of the consequences of chronic or subacute ingestion, absorption and/or inhalation is essential for assessing the parameters of potential hazard of this environmental agent.

PROPOSED COURSE: Absorption of phthalates through the skin or lungs could lead to accumulation in tissues not seen following oral administration. This will be investigated if time permits. The use of di(ethylhexyl) tetrachlorophthalate as a replacement for PCBs should be explored.

PUBLICATIONS

Albro, P.W.: The Metabolism of 2-Ethylhexanol in Rats. Xenobiotica 5: 625-636, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30022-02 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pathogenesis of Hemorrhagic Cyst Disease in Fischer Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	EBCB NIEHS
	R. E. Faith	Staff Fellow	EBCB NIEHS
	D. D. Copeland	Commissioned Officer	EBCB NIEHS
OTHER:	E. E. McConnell	Commissioned Officer	EBCB NIEHS

COOPERATING UNITS (if any)

Darell D. Bigner, M.D., Ph.D., Department of Pathology, Duke University

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

These studies were undertaken to gain insight into the pathogenesis of the condition associated with injection of avian sarcoma virus, known as hemorrhagic cyst disease, and to observe the effects of infection with this virus on immune competence. Hemorrhagic cysts have been shown to become apparent at about 21 days after innoculation of the virus. The cysts appear to develop in lymphoid tissues. Virus infected animals are suppressed in their immune competence, those with cysts more so than those without cysts.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To define the pathogenesis of hemorrhagic cyst disease and evaluate the usefulness of the phenomena as an animal disease model.

METHODS EMPLOYED: Neonatal Fischer rats are inoculated intracranially with the Bratislava-77 strain of avian sarcoma virus (B-77-ASV). The evolution of hemorrhagic cysts is studied by sequential autopsy and fine dissection of cysts. Morphologic features are studied by light microscopy, transmission electron microscopy, and scanning electron microscopy. Cyst fluid is studied by conventional clinical pathologic methods. Cell mediated immunity is being assessed in vitro by the response of thymic or splenic cell cultures to the mitogens Conconavalin A or phytohemagglutinin and in vivo by delayed hypersensitivity responses as measured by tuberculin reactions. Humoral immunity is being assessed by passive hemagglutination to measure antibody response to injected antigens.

MAJOR FINDINGS: All rats were inoculated with virus on the first post natal day. Hemorrhagic cysts begin appearing as early as 21 days and continue to develop for several months. When animals are injected intracranially cyst most commonly develop in the superficial lymph nodes of the neck and the deep lymph nodes of the retroperitonium. One group of animals was injected in the hind leg. Cysts in these animals developed in the draining lymph nodes and in the spleen, thymus, and parathymic lymph nodes. Morphologically the cysts are lined by endothelium and appear to be multivisicular and may contain granulation tissues. The fluid within the cyst is high in protein (2.62-6.34 g/dl) and contains both red blood cells (2.6-34.5 X 10^6/ml) and white blood cells (3.7-27.1 X 10^3/ml) greater than 90% of which are lymphocytes.

Virus injected animals show a suppression of splenic and thymic lymphocytes to stimulation with the mitogens Conconavalin A and phytohemagglutinin. This suppression is more pronounced in animals with cysts than in those without cysts. Delayed hypersensitivity responses are suppressed in virus inoculated animals, cyst bearing animals being more suppressed than non-cyst bearing animals. Virus inoculation also suppresses antibody responses. Again the animals with cysts are more suppressed than are those without cysts.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hemorrhagic cyst disease is a phenomena without known parallel in comparative medicine. Because of the implications that immune parameters are involved in the pathogenesis of the disease the disorder may prove to be a useful and unique model for the investigation of effects of environmental agents in immunologically compromised animals.

PROPOSED COURSE: To publish data generated to date and probably discontinue the project.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30024-02 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Primate Disease Investigation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. McConnell	Commissioned Officer	EBCB NIEHS
OTHER:	J. R. Hass	Staff Fellow	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

Investigation of monkey (Macaca mullata) mortalities at the breeding facility in Perrine, Florida suggested the causative factor(s) to be cyclic chlorinated hydrocarbons, probably polychlorinated biphenyl intoxication. Low serum Vitamin A levels and the pattern of epithelial lesions suggest that this vitamin may be in part involved in the patnogenesis of the syndrome.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To investigate and define a disease syndrome affecting the Rhesus monkey breeding colony at Perrine, Florida since it resembles polychlorinated biphenyl (PCB) intoxication. If possible, to determine the etiology.

METHODS EMPLOYED: On site investigation of the problem. Epidemiological investigation of the disease syndrome with comparison to similar problems at other primate facilities. Necropsy and clinicopathologic determinations on affected animals. Radiographic and histopathologic work-ups on necropsied animals. Spectrophotometric analyses of animal tissues and suspect environmental compounds.

MAJOR FINDINGS: Monkeys in certain cages are more affected than in other areas. The disease syndrome is confined to epithelial tissues with lesions characteristically found in the skin, toenails, eyelids, oral cavity, facial bones, salivary glands, mammary gland, stomach and colon. The essential lesion is metaplasia and/or hyperplasia resembling vitamin A deficiency. Liver and serum Vit. A levels were lower than those in similar age Rhesus monkeys from a different geographic local. These findings suggest chronic toxicity by a compound such as a polycyclic chlorinated hydrocarbon whose effect is mediated, at least partially, through a defect in Vit. A metabolism. Although no significant amounts of PCB's were found in animal tissue, very high levels were measured in surface cement samples from suspect areas. Additional animal tissues are being analyzed to evaluate this apparent discrepancy.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Primate breeding facilities are becoming progressively more important as foreign suppliers of these animals diminish. The usefulness of the facility depends on determining and hopefully eliminating the cause of the problem. The high levels of PCB's in the surface cement samples were attributed to the sealant covering. It was not determined whether the sealant was contaminated prior to its use or after application. However, based on this finding the surface was sanded and retreated with a different covering. Although insufficient time has elapsed to made a definitive statement, the problem appears to have been remedied.

PUBLICATIONS

The responsible official at Perrine is presently completing a paper on the subject to be submitted to Laboratory Animal Science.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30026-02 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Histopathologic Effects of Euthanasia on Laboratory Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Feldman	Veterinary Medical Officer	EBCB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The histopathologic changes caused by various methods of euthanasia were studied in rats, mice, guinea pigs and rabbits. Lesions resulting from a particular method of euthanasia were consistent from species to species. Each method studied affected the lungs to some degree, ranging from mild congestion of alveolar capillaries to marked intramural edema of pulmonary arteries and alteration of vascular permeability. Euthanasia of experimental animals by overexposure to CO_2, or intraperitoneal injection of concentrated sodium pentobarbital seemed most suitable for pulmonary studies. Decapitation (mice, rats, guinea pigs), cervical dislocation (mice), CO_2 and intracardial injection of sodium pentobarbital were more suitable for examination of abdominal viscera.

PROJECT DESCRIPTION

OBJECTIVES: To determine the histopathologic changes that result from various methods of euthanasia in mice, rats, guinea pigs and rabbits.

METHODS EMPLOYED: Groups of 4 animals of each species were killed according to commonly employed methods which included decapitation (mice, rats, guinea pigs), cervical dislocation (mice), CO_2, air embolism (rabbits) and various parenteral injections of sodium pentobarbital. Animals were necropsied immediately after death and tissues were processed for microscopic examination.

MAJOR FINDINGS: Most methods of euthanasia allowed good visualization of abdominal viscera, except concentrated sodium pentobarbital injected intraperitoneally. This drug was caustic to the serosal surface of underlying organs and caused necrosis of capsular and subserosal cells. Most methods of euthanasia caused variable pulmonary vascular congestion but usually the lung architecture was not distorted. Euthanasia with CO_2 or sodium pentobarbital injected intraperitoneally or intravenously affected the lungs minimally. Concentrated sodium pentobarbital injected into the right ventricle of the heart caused hemolysis of erythrocytes and edema of both pulmonary arterial walls and alveoli.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The method of euthanasia merits careful deliberation in the design of experiments. If histopathologic interpretation must be made, it is important that euthanasia does not distort cellular architecture.

PROPOSED COURSE: This project has been completed and the data is being compiled for publication.

PUBLICATIONS

Feldman, D.B. and Gupta, B.N.: Histopathologic Changes in Laboratory Animals Resulting from Various Methods of Euthanasia. Lab. Anim. Sci. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30028-05 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Evaluation of a Captive-Born and Reared Colony of Opossums

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. B. Feldman Veterinary Medical Officer EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

A closed breeding colony of Virginia opossums is maintained to provide neonates for research purposes. Opossums are bred during the 4 month breeding season (January-April) by establishing natural matings within a group of 2-3 females to 1 male. Mortality is high among males who are killed by the females. In 1975 the conception rate was 43% (13 litters from 30 females). Thus far, in 2 months, 7 litters have been born to 49 females (14% conception rate).

PROJECT DESCRIPTION

OBJECTIVES: To evaluate the physiologic, pathologic and reproductive parameters of a "closed" colony of captive-born opossums.

METHODS EMPLOYED: During the 1975 breeding season (January-May), opossums were bred by conventional means i.e. natural matings within a group of 2-3 females:1 male.

MAJOR FINDINGS: A total of 13 litters were born as a result of breeding 30 females (43% conception rate). The number of young totaled 100 of which 58 survived to weaning age. 58% survived. The average litter size was 7-6. In 1976, 49 females were conventionally bred to maintain the nucleus colony. Seven litters have thus far been born (14% conception).

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This colony has been monitored from year to year as a feasible means of providing sufficient animals for research. During the past year research utilizing the opossum has been considerably reduced and the concomitant demands for neonates has lessened. Because of the value of those opossums, the colony will probably be perpetuated but only a minimal number of breeding opossums will be maintained from year to year. In the event of increased demand, the colony can be expanded.

PROPOSED COURSE: Continued monitoring of the colony.

PUBLICATIONS

Feldman, D.B. and Ross, P.W.: Methods for Obtaining Neonates of Known Age from the Virginia Opossums (*Didelphis marsupialis virginiana*). *Lab. Anim. Sci.* 25(4): 437-439, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30030-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Defined Diet Related Hydronephrosis and Hydroureter

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Faith	Staff Fellow	EBCB NIEHS
OTHER:	D. B. Feldman	Veterinary Medical Officer	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	C. A. Kimmel	Senior Staff Fellow	ETB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

Initially a high incidence of hydronephrosis and hydroureter was observed in CD rats fed a marginally zinc deficient defined diet in which sucrose was the sole carbohydrate. These studies were undertaken to elucidate the etiology of the urinary tract pathology. Presently sources of carbohydrate and zinc levels in defined diets are being investigated in relation to urinary tract pathology.

PHS-6040

PROJECT DESCRIPTION

OBJECTIVES: To determine the singular or combined effect of a carbohydrate source and zinc deficiency on the development of urolithiasis, hydroureter, and hydronephrosis in the rat.

METHODS EMPLOYED: Three week old CD rats, both male and female, were randomly assigned to various diet groups. Diets employed to date have differed in zinc content and in sugar source (either sucrose or dextrose). Body weight gains, food consumption, and water consumption were monitored three times weekly. At the end of three weeks the animals were sacrificed and complete necropsies performed.

MAJOR FINDINGS: Rats fed diets in which the sole sugar source is sucrose develop a high incidence of urinary tract lesions. There was a sex difference in these rats in that the incidence of urinary tract pathology in males was 73.8% while the incidence was only 26.3% in females. The zinc levels employed appeared to have no effect on the incidence of urinary tract pathology.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many studies at this Institute and other institutions require the use of defined diets. It is, therefore, of importance to know the effects of defined diets on experimental animals, especially if one or more components of the diet produce pathological changes. In addition, pathological changes that are diet related are of interest from a nutritional point of view.

PROPOSED COURSE: Further investigations planned include: repeating these studies in one or more other strains of rats in order to develop a feel for strain susceptibility; investigation of a diet utilizing a sugar source of fructose and dextrose; and investigation of diets utilizing other disaccharides. The data already obtained is being prepared for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30031-01 EBCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Toxicity of Selected Symmetrical Hexachlorobiphenyl Isomers in Male Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Biocca	Visiting Fellow	EBCB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	K. Chae	Staff Fellow	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Five-week-old male mice were fed: (I) 0.3, 1, 3, 10, 30, 100 and 300 ppm of 3,4,5,3',4',5'-hexachlorobiphenyl (HCB); (II) 10, 30, 100 and 300 ppm of 2,4,5,2',4',5'-HCB; (III) 10, 30, 100 and 300 ppm of 2,3,6,2',3',6'-HCB; and (IV) 10, 30, 100 and 300 ppm of 2,4,6,2',4',6'-HCB. Surviving mice were sacrificed at 28 days. HCB levels in adipose tissue and liver were determined. There were variations among the isomers as to dose and pathologic effects. Isomer (I) showed the greatest effect of those studied on mortality, body weight gain, liver, thymus, spleen, and testes; it also attained the highest tissue concentration. It was the only isomer which produced porphyrin accumulation. The decreasing order of overall toxicity was I>>IV>II>III.

PROJECT DESCRIPTION

OBJECTIVES: To determine the toxicity of different symmetrical isomers of hexachlorobiphenyl in the mouse.

METHODS EMPLOYED: Five week-old male mice were fed (a) 3,4,5,3',4',5'-hexachlorobiphenyl (HCB) @ 0.3, 1, 3, 10, 30, 100 and 300 ppm; (b) 2,4,5,2',4',5'-HCB; (c) 2,3,6,2',3',6'-HCB; and (d) 2,4,6,2',4',6'-HCB each @ 10, 30, 100 and 300 ppm incorporated into feed. The mice were fed continuously for a period of 28 days. Mortality, body weight gain and weights of liver, spleen, thymus and testes were recorded. Necropsy examination was performed on moribund and surviving mice. Levels of HCB's in adipose tissue and liver were also determined.

MAJOR FINDINGS: Out of 4 isomers studied, 3,4,5,3',4',5'-HCB was the most toxic in mice. LD_{50} was determined to be 86.8 ppm of 3,4,5,3',4',5'-HCB on continuous feedings for 28 days in male mice. There was an abnormal accumulation of porphyrin in the liver, bones and teeth as denoted by red fluorescence under long wavelength ultraviolet light. Moderate to marked fatty metamorphosis and single cell necrosis of hepatocytes were observed microscopically. There were moderate to marked depletion of lymphocytic type cells from the lymphoid organs. The mice treated with 2,4,6,2',4',6'-HCB at concentrations of 300 ppm had focal necrosis and mineralization of myocardial muscle fibers.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polychlorinated biphenyls (PCBs) are a well-known class of chemicals which have been industrially produced in several countries for over 44 years. They are very persistent as environmental contaminants and pollutants. This study was undertaken to pursue a systematical investigation in a mammalian species of the toxicity and biological effects of some selected symmetrical hexachlorobiphenyls.

PROPOSED COURSE: To study the long-term toxicity of different isomers of PCBs on very low concentrations in the diet.

PUBLICATIONS

Biocca, M., Moore, J.A., Gupta, B.N. and McKinney, J.D.: Toxicology of Selected Symmetrical Hexachlorobiphenyl Isomers: Biological Responses in Chicks and Mice. National Conference on Polychlorinated Biphenyls, Chicago, Ill. (March, 1976): 89-98.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30032-01 EBCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biological Effects of 3,4,5,3',4',5'-Hexachlorobiphenyl in the Chick

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
OTHER:	K. Chae	Staff Fellow	EBCB NIEHS
	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Comparative Biology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

3,4,5,3',4',5'-hexachlorobiphenyl (HCB) was fed to 1-day-old chicks at varying concentrations from 0.01 to 60.0 ppm in feed for 21 days. The LD_{50} appears to lie between 1 and 2 ppm of HCB in feed. All chicks died betwene 6 and 18 days fed HCB at a concentration of 3.0 ppm and more. A cumulative total daily intake of HCB from 0.12 mg to 0.32 mg at 3.0 ppm level in feed caused 100% mortality. Ascites, hydropericardium and subcutaneous edema developed in all chicks fed 3.0 ppm and above. Red fluorescence under UV light denoting abnormal accumulation of porphyrin was observed in the mucosal layer of gizzard, famur, sternum, skull, liver and cecum. Atrophy of bursa and thymus, necrosis and inflammatory changes in the mucosal layer of gizzard, and necrosis of hepatocytes were observed microscopically. 3,4,5,3',4',5'-HCB appears to be the most toxic isomer of PCB tested in avian species.

PROJECT DESCRIPTION

OBJECTIVES: To determine the LD_{50} and biologic effect of 3,4,5,3',4',5'-hexachlorobiphenyl in the chicken.

METHODS EMPLOYED: One day-old male White Leghorn chicks were fed 3,4,5,3',4',5'-HCB at 0.0 (control), 0.01, 0.05, 0.1, 0.5, 3.0, 10.0, 30 and 60 ppm for 21 days. Each chick was individually tagged and body weight was recorded three times a week. Daily food intake, body weight gain and weights of liver, spleen and heart were recorded. Complete necropsy examination was performed on dead, moribund and surviving chicks. Different organs were also examined under the long wavelength ultraviolet light for the presence of abnormal porphyrin accumulation. Peritoneal fluid and small intestine from selected treated and control chicks were cultured for the presence of pathogenic bacteria or mycoplasma.

MAJOR FINDINGS: There was a relative increase in the weights of liver and heart in 3.0 ppm dose level. The weights of liver and heart decreased significantly ($P<0.05$) in higher dose levels (30 and 60 ppm). There was 100% mortality in 3 ppm and above dose levels, and 30% of the chicks died within 21 days of feeding in 0.5 ppm level. The LD_{50} for 3,4,5,3',4',5'-HCB appears to be somewhere between 1 and 2 ppm. The average period of death was 11 days in 60 ppm and 15 days in 3 ppm. The total amount of 3,4,5,3',4',5'-HCB to produce mortality in the chick varied from 0.12 to 0.32 mg in 3 ppm group and 1.2 to 1.9 mg in 60 ppm. All chicks died between 6 and 18 days.

The average daily food intake was inversely proportional to the concentration of HCB in the diet i.e. higher the concentration of HCB in diet, lower the food consumption. The consumption of HCB-treated food in all groups of chicks was less compared with the controls at every time point. Similarly, the body weight gain in all HCB-treated chick was also lower compared with the chick on control diet.

Several microorganisms, Escherie coli, Enterococcus sp., and Klebsiella sp. were isolated consistently from the peritoneal fluid of treated moribund or dead chicks. No microorganism was cultured from the peritoneal of cntrol chicks. E. coli, Enterococcus sp. and Klebsiella sp. were also isolated from the small intestines of treated and control chicks. All samples were negative for mycoplasma.

Red fluorescence under UV light was not observed in any organ of chicks treated with 3 ppm or less of HCB at any time point of observation. Mucosal layer of the gizzard fluoresced as early as 9 days in 60 ppm group and 88% (7 out of 8) were positive for abnormal porphyrin accumulation. The thick muscular layer of gizzard did not fluoresce. The following other organs in the order were found positive for porphyrin under UV light: Femur, sternum, skull, liver, and cecum.

All chicks fed HCB at 3 ppm or more developed subcutaneous edema and had gelatinous appearance of subcutaneous adipose tissue. These were more pronounced in the neck, shoulder and upper thigh regions. The abdomen was markedly distended due to fluid accumulation in the peritoneal cavity. The thymus appeared congested and markedly involuted. Hydropericardium, paleness of liver, and loss of visceral and subcutaneous adipose tissue were consistently observed. The fluid appeared turbid whereas the pericardial fluid was clear.

Remarkable pathologic changes in different organs at all dose levels are summarized in Table 1.

Histopathologic changes were observed in gizzard, thymus and bursa consistently at 3 ppm and above. Microscopic changes in the gizzard was characterized by marked degeneration, necrosis, fibrinoid and cystic changes, purulent exudation and presence of numerous bacterial colonies in the mucosal layer.

Marked depletion of lymphocytic type cells were observed in lymphoid organs primarily in thymus and bursa. There was necrosis, cystic changes filled with mucus, and proliferation of reticuloendothelial cells in the lymphoid follicles of the bursa in most chickens.

Single cell necrosis of hepatocytes, dilation of sinusoids and slight to moderate congestion of liver were also observed. Dilation of renal tubules, atrophy of glomeruler tufts, and hemorrhage in colon and cloaca were noted occasionally. The mucosal layer of the tongue in some chicks had some degenerative changes.

There was slight to moderate involution of bursa and thymus in 0.5 ppm level. All other organs were within normal range in 0.5 ppm and lower dose levels.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Polychlorinated biphenyls (PCBs) are a well-known class of chemicals which have been industrially produced in several countries for over 44 years. They are very persistent as environmental contaminants and pollutants. To develop a hypothesis which predicts biological effects based on chemical structure, a characterization of specific isomer effects is needed.

PROPOSED COURSE: To determine the pathogenesis and biologic effects of different isomers in avian and mammalian species.

Table 1 - Summary of Major Pathologic Changes in the Chick Given 3,4,5,3',4',5'-Hexachlorobiphenyl for 21 Days

Concentration (PPM)	Liver	Thymus	Bursa	Gizzard (Mucosal layers)	Tongue	Ascites
0.01	None	None	None	None	None	None
0.05	None	None	None	None	None	None
0.10	None	None	None	None	None	None
0.5	Slight	Moderate	Marked	None	None	None
3.0	Marked	Marked	Marked	Moderate	Moderate	Marked
10.0	Slight	Marked	Marked	Marked	Marked	Marked
30.0	Slight	Marked	Marked	Marked	Slight	Marked
60.0	Slight	Marked	Marked	Marked	Slight	Marked

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30033-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Factors in Drinking Water Affecting Experimental Animal Reproduction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
	J. R. Hass	Staff Fellow	EBCB NIEHS
	R. R. Maurer	Senior Staff Fellow	ETB NIEHS
OTHER:	P. W. Albro	Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)
Environmental Toxicology and Biometry Branches, NIEHS
City of Durham, Water Treatment Plant
UNC Dept. of Environ. Sciences and Engineering, Chapel Hill, NC

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The $CHCl_3$ levels in the NIEHS drinking water were determined for a 9 month period. These levels were found to inversely correlate with apparent reproductive failures in certain experimental animals in the Institute. A sampling of the total organics revealed the presence of 51 different partially halogenated hydrocarbons ranging from C_1-C_5. Chemical oxygen demand determinations for the months of March and August (considerably different $CHCl_3$ levels found) indicate that there is little difference in total organics concentration. These findings suggest that higher concentrations of the larger organic molecules found in tap water during the colder months due to less extensive degradation on chlorination could be a minor acute toxicity problem in regard to reproduction.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To identify the possible causative factors in drinking water responsible for apparent reproductive failure in experimental animal colonies.

METHODS EMPLOYED: Water samples were collected twice a week taking care to completely fill and tightly seal the sample bottles. These samples were analyzed by means of electron capture gas chromatography using a Carbowax 400/Porasil Durapak column. An internal standard of CH_2Cl_2 was employed.

Total organics were determined by current practive carbon and resin adsorption techniques followed by gas chromatography/mass spectrometric analysis. Chemical oxygen demands (CODs) were determined using the dichromate method.

MAJOR FINDINGS: The levels were found to range from 150 ppb to 1500 ppb, rising fairly uniformly from February, 1975 through August, 1975 and then declining again. The $CHCl_3$ levels correlated directly with the "chlorine demand" reported by the Durham water plant but inversely with marked reproductive failure in experimental animals. Since $CHCl_3$ is probably a good marker compound for relative levels of simple halogenated compounds, chlorination of the drinking water is not implicated as the cause of the breeding failure. However, less extensive degradative chlorination during the colder months would implicate the larger natural product organics which would include endotoxins.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is increasing concern over the practice of chlorinating drinking water. Since the drinking water is implicated as a possible source of apparent reproductive failure at NIEHS, it may be a matter of public health concern to study the chemistry of aqueous chlorination and the toxicology of the encompassing products.

PROPOSED COURSE: To complete $CHCl_3$ level determination for the remaining months. To determine the nature of the major large organic molecules present in finished water.

PUBLICATIONS

McKinney, J.D., Maurer, R.R., Hass, J.R. and Thomas, R.O.: Possible Factors in the Drinking Water of Laboratory Animals Causing Reproductive Failure. Proceedings of Symposium on Identification and Analysis of Organic Pollutants in Drinking Water. First Chemical Congress of the North American Continent, Mexico City, November, 1975. (Chapter 25 of book to be published by Ann Arbor Science Publishers, Inc.).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30034-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chemistry of Chlorinated Aromatic Compounds and Their Environmental Transformation Products

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. A. Levy	Research Chemist	EBCB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The synthesis of environmentally important chlorinated aromatic compounds and oxygenated chlorinated aromatics were investigated. Spectra of pure materials as well as those obtained from synthesis mixtures were collected and found use in the identification of such classes of compounds and their metabolites.

PROJECT DESCRIPTION

OBJECTIVES: To develop rational synthetic routes to chlorinated biphenyls, chlorinated dibenzofurans, prefurans and their metabolites, especially the assumed reactive intermediate arene oxides (oxepins) and penultimate hydroxylated derivatives. To investigate the chemical and physical properties of these compounds and their interaction-reaction products, which will aid in identifying them in the environment and assessment (rate and extent) of reactivity with biological entities.

METHODS EMPLOYED: Synthetic techniques, organometallic reagents and catalysis, high pressure reactions, photochemical processes, functional group transformations. Mass spectroscopy, other spectroscopic methods (IR, NMR), chromatography (column, glc, liquid).

MAJOR FINDINGS: The mass spectra of a large number of polychlorinated biphenyls were obtained and their fragmentation as a function of substitution was found. This work has been of value in the structural elucidation of unknown chlorinated biphenyls.

The Diels-Alder cycloaddition reaction and subsequent functional group manipulations has yielded derivatives only one oxidation step removed from the highly desired biphenyl oxide.

Work on the synthesis of chlorinated dibenzofurans by an approach which introduces the carbon carbon bond joining the two aromatic nuclei as the last step has not yielded pure compounds. However, mass spectra, useful in the identification of these compounds, have been obtained from the mixtures so produced.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The commercial PCB's are known to be complex mixtures and are recognized to be of major environmental importance due to their widespread ecological distribution and their concentration in the food chain. Furthermore, chlorinated dibenzofuran impurities found along with PCB and/or produced from PCB by natural processes are known to be more toxic. The availability of the unique isomers of these classes of compounds and their probable metabolites would permit more definitive biological and toxicological studies.

PROPOSED COURSE: To continue the synthesis of individual isomers of PCB's and dibenzofurans as required as well as their potential or actual metabolites such as arene oxides and hydroxylated derivatives. Continuing attention is to be focused on the use of the cycloaddition reaction and associated sequence mentioned above. The synthesis of dibenzofurans by way of organometallic intermediates may be explored.

PUBLICATIONS

Levy, L.A., Oswald, E.O.: The Effect of Ortho Substitution on the Mass Spectral Fragmentation of Polychlorinated Biphenyls. Biomedical Mass Spectroscopy, 0000, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30035-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chemical Techniques and Spectroscopic Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
	P. W. Albro	Research Chemist	EBCB NIEHS
	J. R. Hass	Staff Fellow	EBCB NIEHS
	L. A. Levy	Research Chemist	EBCB NIEHS
	K. Chae	Staff Fellow	EBCB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

Midwest Research Institute (Procurement Contract with NCI No. NO1-CP-33387. Supplemented by NIEHS). Others as necessary.

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

This general project has as its objective to employ chemical techniques, both analytical and synthetic, and spectroscopic methods, in particular gas chromatography/mass spectrometry (GC/MS), nuclear magnetic resonance spectroscopy (NMR) and high pressure liquid chromatography (HPLC), to the preparation, determination and characterization of compounds and classes of compounds of general interest to the Institute and specific interest to individual investigators. In general compounds covered by this study will include those for which adequate techniques and methods are already available or can be adapted.

PROJECT DESCRIPTION

OBJECTIVES: To employ analytical and synthetic techniques and methods to the preparation, determination, and characterization of compounds and compound classes of general interest to the Institute and specific interest to individual investigators. In general, compounds covered by this study will include those for which adequate methodology is available either in the literature or otherwise, but may require some trial and error period to adapt.

METHODS EMPLOYED: The analytical methods emphasized in this study are GC/MS, NMR or fourier transform NMR and HPLC. A variety of synthetic techniques are employed. Other special methods will be employed where necessary or as other instrumentation becomes available.

MAJOR FINDINGS: The GC/MS facility analyzed about 300 different samples upon request of which about 60% was in support of various projects within the chemistry section, about 23% was for other Institute requests and about 15% was in search for PCBs and the identification of the monkey disease factor (see project number Z01 ES 30024-02 EBCB).

The NMR facility handled over 50 different samples including a variety of chemical standards and biological metabolites and in many observed both ^{1}H and ^{13}C nuclei using ^{1}H decoupling techniques. Again a large number of these samples were generated from other projects within the section. For example, preliminary studies with diazonium conjugates of amino acids have indicated that ^{13}C-NMR may be useful in the qualitative analysis of haptens and antigens in immunochemistry method development. Other work with ^{13}C-enriched chlorinated aromatics has indicated that ^{13}C-labeling may be a nice complement to ^{14}C-labeling in metabolism studies. Other ^{13}C-labeled compounds to be investigated include some specific ^{13}C-labeled benzo[a]pyrene oxygenated derivatives currently being synthesized.

About 23 requests for HPLC analysis were made and only one of those was from outside the Chemistry Section.

At least eleven other requests were made for analytical methods which required specific approaches. These ranged from specific analyses for various enzymes, urinary calculae, vitamins, metabolites and fatty acid esters to determinations of purity and radiopurity of unlabeled and ^{14}C-labeled chlorinated hydrocarbons.

An attempt was made to determine the purity and nature of the impurities in commercially available dimethyhydrazine (DMH) dihydrochloride to be used in carcinogenic studies. The DMH was shown to decompose slowly on storage in solution to form yellow and UV-absorbing products. The decomposition is faster in ethanol or ether than in aqueous solutions, and is pH-sensitive. Decomposition products and their carcinogenic potential have not been conclusively determined as yet.

The more difficult synthetic requests include 8(N-fluroenylacetamido)-2'-deoxyguanosine and specifically (^{3}H and ^{14}C) labeled prostaglandin derivatives requiring high chemical purity and radiopurity and known and unique stereochemistry.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The preparation, determination and characterization of various compounds and compound classes of general interest to the Institute will facilitate the successful elaboration of their chemistry and biochemistry and permit more definitive biological and toxicological studies.

PROPOSED COURSE: Chemical techniques and methodologies will continue to be employed as needed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30036-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Enzymatic Reductive Dechlorination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Chae	Staff Fellow	EBCB NIEHS
OTHER:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The reductive dechlorination potential of an in vitro system (rat liver homogenate) was investigated to aid in elucidating the role of metabolic dechlorination in toxicity. The chlorinated polycyclodiene pesticide dieldrin was chosen as a model substrate due to the availability of the various dechloro products which might be expected. Preliminary findings suggest that dieldrin may require NADPH and anaerobic conditions to effect dechlorination.

PHS-6040

PROJECT DESCRIPTION

OBJECTIVES: To investigate the relative propensities of selected chlorinated hydrocarbons toward biological dechlorination and establish the preferred mechanism(s). To compare these results with those obtained from biomimetric models for the dechlorination process(es) (see project number Z01 ES 30017-03 EBCB).

METHODS EMPLOYED: Subcellular fractions were prepared from rat liver homogenates by standard procedures. ^{14}C-Dieldrin and unlabeled dieldrin were used as initial substrates since the expected dechloro products were already available from chemical and photochemical studies. Various cofactors were incorporated and the products determined using thin layer and gas chromatography and autoradiography.

MAJOR FINDINGS: Preliminary findings have indicated that the dechlorination of dieldrin may require NADPH and anaerobic conditions. The product characterization and cofactor requirements are currently being investigated.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUE: The ultimate fate of many chlorinated hydrocarbons may be a function of dechlorination propensity. The dechlorination process(es) may be both toxifying and detoxifying depending on the nature of the intermediary and terminal metabolites. If relative propensities toward dechlorination could be determined, they may be of predictive value for determining persistence and toxicity.

PROPOSED COURSE: This project has just begun and will probably receive little attention in the immediate future due to higher priority projects. However, if time permits, it will be continued as outlined above.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30037-01 EBCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Negative Ion Chemical Ionization Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. R. Hass Staff Fellow EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Chemistry Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The NIEHS high pressure mass spectrometer is undergoing modification to permit the formation and observation of either positive or negative ion beams. These modifications are nearing completion.

PROJECT DESCRIPTION

OBJECTIVES: To develop a negative ion chemical ionization mass spectrometry (NICIMS) capability at NIEHS. To study the NICIMS of chemicals of environmental interest. To develop analytical methodology exploiting NICIMS.

METHODS EMPLOYED: The NIEHS high pressure mass spectrometer has been modified to permit the production and observation of negative ion beams. These modifications include a Townsend discharge source and the installation of necessary high vacuum feed throughs and isolation preamplier so that A.C. signals can be extracted from up to 6 KV D.C. Modifications to the ion optics and the electron multiplier are nearing completion which will permit the mass analysis and detection of negative ion beams.

MAJOR FINDINGS: There are no findings to report at this date since the first year of the study was devoted to rather extensive instrument modifications.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many chemicals of environmental health interest contain halogen atoms. These halogens have a high electron capture cross-section and can thus be expected to have a high sensitivity for NICIMS. Since the majority of naturally occuring biological compounds do not have a comparable electron capture cross-section, it can be anticipated that a substantial increase in signal/biological noise will be realized in the analysis of the halogenated compounds in biological systems.

PROPOSED COURSE: Practical applications of the method are proposed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30038-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Toxicity of Dibenzofurans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
OTHER:	E. E. McConnell	Commissioned Officer	EBCB NIEHS
	M. Harris	Biological Laboratory Technician	EBCB NIEHS

COOPERATING UNITS (if any)

Kun Chae, Ph.D., Chemistry Section, EBCB
Hazleton Research Laboratories, Vienna, Va.

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to evaluate various dibenzofurans with regard to the following parameters (1) Dose response; (2) Lethal dose; (3) Clinical signs; (4) Clinical Pathology; and (5) Anatomic Pathology in quinea pigs, mice and monkeys.

PROJECT DESCRIPTION

OBJECTIVES: Using pure halogenated dibenzofurans, determine the dose required to produce a toxic or lethal response in laboratory animals. Compare the LD_{50} and lesions produced by various isomers within an animal species and between species, i.e., mice and guinea pigs. Evaluate hematology and clinical chemistry parameters.

METHODS EMPLOYED: Male guinea pigs and/or mice were given single or multiple doses of various dibenzofurans [2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,7,8-tetrabromodibenzofuran (TBDF), and 2,3,4,7,8-pentachlorodibenzofuran (PCDF)] orally. Standard techniques were used to evaluate the objectives.

MAJOR FINDINGS: While much of the data is not completed to date these compounds appear to be extremely toxic with the LD_{50} in guinea pigs being 5-10 µg/kg for TCDF and 3-10 µg/kg for PCDF or about 3 to 5 times less toxic than their dioxin counterpart. The brominated form (TBDF) was of about the same toxicity (3-10 µg/kg) as TCDF. The lesions produced by these compounds were similar in a given species of animal. However, a distinct difference was noted between species, i.e. guinea pigs and mice. Thymic and splenic atrophy were noted in both species. Toxic hepatitis with porphria and vascular lesions (hemorrhage and edema) were prominent features in mice. These were not found in guinea pigs. In contrast, testicular and bone marrow atrophy along with adrenal cortical hemorrhage and hyperplasia of the renal pelvic epithelium were more characteristically found in guinea pigs. Overall, the lesions were comparable to those produced by dioxins in the same species of animal.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Halogenated dibenzofurans may occur in association with useful chlorinated cyclic compounds. In addition, there is evidence that they were contaminants of polychlorinated biphenyls and may have contributed in the Yusho accident. The pattern of toxicity associated with most of the isomers is not known. These facts must be known if assessment of the environmental health implication of the materials which contain these compounds is to be made.

PROPOSED COURSE: To be continued.

PUBLICATIONS

Moore, J.A., Gupta, B.N., and Vos, J.G.: Toxicity of 2,3,7,8-Tetrachlorodibenzofuran: Preliminary Results. National Conference on Polychlorinated Biphenyls, Chicago, Ill. (March, 1976).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30039-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Monkeys (Macaca cynomolgus)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. McConnell	Commissioned Officer	EBCB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	D. Dalgard	Project Coordinator	Hazleton Labs, Vienna, VA

COOPERATING UNITS (if any)
K. Chae, Ph.D., Chemistry Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study was to establish the oral lethal dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin in monkeys (Macaca cynomolgus). In addition to evaluate the following parameters (1) clinical signs; (2) clinical pathology; and (3) anatomic pathology.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: Using pure 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), determine the LD_{50} and evaluate the lesions in monkeys (Macaca cynomolgus).

METHODS EMPLOYED: Monkeys (at Hazelton Labs) were given a single dose of TCDD in corn oil orally. Parameters of evaluation included clinical observations, hematology and clinical chemistry (pre and post exposure), macro and histopathology.

MAJOR FINDINGS: The LD_{50} was less than 70 µg/kg, probably around 50 µg/kg. Time to death was dose dependent (28 ± 5 days at 350 µg/kg and 42 ± 5 days at 70 µg/kg). The clinical appearance of the animals was characterized by weight loss, focal alopecia, blepharitis, acne, abnormal nail growth and progressive weakness. Hematology and clinical chemistry data is not complete as of March 1, 1976. Necropsy and histopathologic examination revealed metaplastic and hypertrophic lesions in sebaccous glands, especially in the eyelids and ear canals. There was marked depletion of lymphoid elements, especially in the thymus where only a few medullary cells remained. The stomach, duodenum and colon showed early cystic and hypertrophic changes in the crypts. The histology of the liver varied with time. Early lesions were characterized by dilatation and hypertrophy of the bile ducts which extended into the gall bladder and common bile duct. In addition to this, later lesions included focal necrosis with inflammation, bile duct proliferation and mild megalocytosis. Hyperplasia of the renal pelvic epithelium was also noted.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated dibenzodioxins may occur in association with many useful chlorinated cyclic compounds. The symmetrical tetrachloro isomer is one of the most toxic chemicals known as has been demonstrated in chickens, guinea pigs and small rodents. This primate evaluation is a natural outgrowth of these studies.

PROPOSED COURSE: To be completed this FY.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 30040-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Dose Response to the Carcinogens, 1,2-Dimethylhydrazine (DMH) and Methylazoxymethanol (MAM) in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. McConnell	Commissioned Officer	EBCB NIEHS
OTHER:	J. A. Moore	Supervisory Veterinary Medical Officer	EBCB NIEHS
	R. E. Wilson	Biological Laboratory Technician	EBCB NIEHS

COOPERATING UNITS (if any)

J. Fawkes, Chemistry Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Comparative Biology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.4	0.7

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to establish the oral dose of the carcinogens 1,2-dimethylhydrazine and methylazoxymethanol required to produce a 15 ± 5% incidence of colon cancer in rats and characterize other pathological effects.

PROJECT DESCRIPTION

OBJECTIVES: The "HEW Committee to Coordinate Toxicology and Other Related Programs" has designed a study to establish the biologic consequence from ingestion of asbestos. NIEHS has the responsibility for effecting these protocols. One aspect of the study is to determine if asbestos can act as a co-carcinogen, specifically in the gastrointestinal tract. DMH and MAM are known intestinal carcinogens, however, the dose required to produce a relatively low incidence (15 ± 5%) is not known. The objective of this study is to derive this information and in addition, to critically evaluate any neoplasm produced as to location, size, and morphology.

METHODS EMPLOYED: Five week old male and female Fisher (CDF) rats were given various amounts of either DMH or MAM via gavage. They were dosed every other week for 8 weeks (5 doses). Body weight gain is being monitored. The study will be terminated at 9 to 12 months depending on the incidence of neoplasia and latent period. Complete necropsies will be performed on all animals with emphasis being placed on the gastrointestinal tract. Tumors will be recorded as to their location, number, size and morphology.

MAJOR FINDINGS: There was a small but significant dose related decrease in weight gain in exposed rats. As of March 1, the study has been underway for 5 months with no indication of neoplasia to date. However, the expected latent period is 6-8 months.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Colon cancer is the 2nd leading form of neoplasia in men and 3rd in women. Various studies have indicated that it is probably environmentally related. Suggestions have been made that asbestos may play a role in the causation. The development of a method which would yield a relatively low incidence would serve as a useful model for the study of this important disease.

PROPOSED COURSE: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30041-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Artificial Antigens from Appropriate Hapten Models

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. W. Albro	Research Chemist	EBCB NIEHS
OTHER:	J. D. McKinney	Supervisory Research Chemist	EBCB NIEHS
	K. Chae	Staff Fellow	EBCB NIEHS
	L. A. Levy	Research Chemist	EBCB NIEHS
	L. K. Cho	Visiting Fellow	EBCB NIEHS

COOPERATING UNITS (if any)

R. E. Faith, D.V.M., Comparative Biology Section, EBCB

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Chemistry Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.5	0.8

SUMMARY OF WORK (200 words or less - underline keywords)

Starting from amino derivatives of chlorinated aromatic and other model compounds including chlorinated anilines and chlorinated amino biphenyl ethers, various more reactive derivatives including Schiff bases, malonyl diethyl amides, N-hydroxysuccinamide esters (as activated esters), diazoanilines and the like have been prepared. Attempts have been made to prepare defined products on coupling these derivatives to various proteins. Techniques are being developed for the characterization of the conjugates. Relative effectiveness and desirability of various derivatives as antigenic determinants will be assessed.

PROJECT DESCRIPTION

OBJECTIVES: To prepare suitable and appropriate haptens. To develop coupling procedures, which reproducibly yield defined products, for the attachment of haptens to proteins. To devise purification procedures for the conjugates, and to evaluate the various procedures in terms of simplicity, reproducibility, flexability, general applicability, and degree of extraneous alteration of the proteins.

METHODS EMPLOYED: Synthetic techniques: Condensation reactions; amination; mixed ester formation; reduction; preparation of active esters; convergent synthesis. Chromatography (glc, column, tlc). Spectroscopy (nmr, ir, gc/ms).

Procedures presently under consideration include diazo coupling, activated ester (N-hydroxysuccinamide) transfer, Schiff base addition, Mannich base formation, isobutyl chlorocarbonate activation, and glutaraldehyde cross-linking. Products are characterized by analytical gel filtration, UV-visible spectroscopy, chemical assay of $_{13}$-NH_2, -SH, Ar-OH and indole groups in the derivatized proteins, NMR (^{13}C) spectrometry, and aminoacid analysis.

MAJOR FINDINGS: General active esters for use in coupling to protein were prepared. The stability of the unit linking the hapten nucleus with the activated ester portion was found to be a function of the number of carbon atoms joining the two centers.

Several peculiarities are associated with ether substitution ortho to an aromatic amino group which limit feasible coupling procedures. The Mannich reaction appears to produce only insoluble conjugates. Azo proteins, thus far, have been quite acceptable derivatives.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Immunoassay offers advantages of extreme specificity and sensitivity such that detection of hazardous compounds, mutated proteins, residues, etc. at biologically meaningful levels may be feasible with this approach. Most hapten-protein conjugates synthesized in the past have not been well characterized or reproducible, weaknesses hopefully to be overcome in the present project. Chlorinated diphenyl ether derivatives (dibenzo-dioxins and dibenzofurans) are currently being emphasized since some are exquisitely toxic and may be widespread environmental contaminants.

PROPOSED COURSE: Studies as outlined above will continue. The products from this project (protein conjugates) will be utilized in a separate project for evaluation of antigenicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 30042-01 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Environmental Contaminants on Cardiac Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. W. Van Stee Commissioned Officer EBCB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biology and Chemistry Branch
SECTION
Inhalation Toxicology Section
INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	0.25

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed toward the detection of functional changes in the cardiovascular system associated with the inhalation of anesthetics, fluorocarbons, and brominated compounds as well as energy-related pollutants. The function of isolated, perfused rabbit, rat, and guinea pig hearts will be monitored. Animals may be previously exposed to contaminants followed by excision and perfusion of hearts or the hearts may be exposed acutely in vitro. Myocardial mechanical, electrical, and metabolic activity may be monitored concurrently.

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to assess the cardiac effects from exposure to various environmental agents. At the present time studies will be performed using isolated perfused small animal hearts. Compounds of current interest include low molecular weight halogenated alkanes such as anesthetics, fluorocarbons, and brominated compounds as well as some of the energy related pollutants. In the future we would like to be able to compare in vitro vs. in vivo preparations using surgically implanted energy transducers and detectors.

METHODS EMPLOYED: Two model systems are employed: (1) the perfused, isolated, Langendorff heart preparation; and (2) the superfused isolated papillary muscle preparation. Mechanical activity in the whole heart is monitored using an intraventricular ballon connected to a pressure transducer, and in the papillary muscle using a force transducer. Electrical activity is assessed using surface electrograms or microelectrode recordings. Metabolic activity of the whole heart is determined by monitoring perfusate pH, PCO_2, and PO_2 as it enters the aorta and as it exits the coronary sinus.

MAJOR FINDINGS: This is a new project, and to date the results consists mostly of purchasing and setting up the proper equipment. This has been accomplished and all the equipment either has been calibrated and is functional or is on order.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lung represents a "short circuit" to the heart since inhaled materials which are transferred to the blood are then pumped directly to the heart and to the blood supply to the heart without benefit of passing through any of the detoxification organs such as the liver and the kidney. Thus, it is necessary that we measure the cardiac effects of these agents directly. Many halogenated alkanes are known to be cardiac depressants, causing arrhythmogenic and or negative inotropic effects. Since the cardiac effects could be lethal, it is necessary to establish an understanding of the mechanism whereby these agents cause their cardiotoxicity.

PROPOSED COURSE: Myocardial excitation and contraction and pacemaker activity will be monitored during controlled exposures of isolated perfused hearts to various halogenated alkanes and energy related pollutants. It has been suggested that the myocardial depressant action of haloalkane is a function of the nature of the substrate available for oxidation by the myocardium. This possibility will be investigated for other halogenated alkanes by comparing the susceptibility of the heart to depression by these compounds in the presence of different substrates, chiefly glucose and fatty acids.

PUBLICATIONS

Van Stee, E.W.: Toxicology of Inhalation Anesthetics and Metabolites. Ann. Rev. Pharmacol. Toxicol. 16: 67-79, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80017-05 EBCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Inhalation Toxicity of Compounds Common to Household Aerosols

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. T. Drew	Supervisory Physical Scientist	EBCB NIEHS
OTHER:	B. N. Gupta	Veterinary Medical Officer	EBCB NIEHS
	J. J. O'Neil	Staff Fellow	EBCB NIEHS
	E. W. Van Stee	Commissioned Officer	EBCB NIEHS

COOPERATING UNITS (if any)

Histology Section, EBCB
Environmental Biometry Branch

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.4	1.3

SUMMARY OF WORK (200 words or less - underline keywords)

The inhalation toxicity of commercial aerosol products and separate components of aerosol products are being studied. Animals are exposed to the compounds under dynamic conditions and the toxicity is evaluated using several indices, including organ weights, morphological changes, changes in hepatic and pulmonary enzyme activity and respiratory physiology. Compounds of current interest include aluminum-chloride-hydroxide, oven cleaners, spray starches and fluorocarbon propellants.

PROJECT DESCRIPTION

OBJECTIVES: To determine acute and chronic inhalation toxicity of selected compounds common to household aerosols and to identify any potential synergistic interactions. Compounds previously investigated include Rehydrol ASC (a propylene glycol complex of aluminum-chloride-hydroxide used in aerosol deodorants), "Airwick", hexachlorophene, dichlorodifluoromethane (F-12) a commercial preparation of hair spray, and a commercial spray adhesive. Compounds recently investigated include Micro-dry (another aluminum-chloride-hydroxide compound not complexed with propylene glycol), several commercial preparations of aerosol oven cleaners, F-12, trichlorofluoromethane (F-11) and two commercial preparations of spray starch.

METHODS EMPLOYED: 1. Exposure Techniques: Exposures are carried out under dynamic conditions with contaminant concentrations being monitored frequently during exposures. Aerosols of aluminum-chloride-hydroxide are generated from an aqueous solution with a compressed air-driven nebulizer. Commercial aerosols are ejected into an elutriation chamber which traps the larger particles and then into an exposure chamber. The cans are activated mechanically on a 5-second on - 25-second off cycle. The fluorocarbons are blended with compressed air to the concentration desired and then directed to the chamber or in some cases to an endotracheal tube for direct exposure.

2. Toxicity Evaluation: Several techniques are utilized to determine the effects of exposures. These include histologic preparation of tissue and microscopic examination for pathological or morphological changes; investigation of mixed-function oxidase (MFO) activities of both lung and liver microsomes after animal exposure; assessment of weight gain and organ weights after exposure; investigation of any change in respiratory physiological parameters such as breathing rate and volume, resistance, and static and dynamic compliance; measurement of electrocardiographic and hemodynamic parameters, and myocardial contractility changes after and during exposures; and measurement of deposition and clearance of chemicals in the lung, and body distribution and elimination of chemicals after exposure.

MAJOR FINDINGS: 1. Animal exposure to either compound of aluminum for 3 days causes increases in lung weights in hamsters, rats, and rabbits. Exposure of rats and hamsters to aluminum-chloride-hydroxide at a concentration of 50 mg/m^3 for 6 hrs/day, 5 days/week for 30 days induces permanent changes. In rats a year later, the lungs of exposed animals are obviously larger and weigh about twice as much as lungs from air-exposed control animals. Histologically, lesions are present which may precede emphysema. The half-like of aluminum in the lungs is about 30 days in hamsters and 60 days in rats.

2. Oven Cleaners. Of 5 brands initially investigated, 3 were chosen for study. Rats and hamsters were exposed to 2 cans per day for 1, 2, 3

or 4 days and sacrificed 14 days later. Other animals were exposed to the output of 2 cans and sacrificed 1, 4, 7 or 10 days later. At sacrifice, several organs were weighed and after preparation were examined for morphological changes. No evidence of toxic effects of exposure to any of the 3 brands chosen was seen -- either in weight gain, organ-to-body weight ratios or morphological changes in lung or other organs. Similar negative findings were seen with the spray starches.

3. Fluoro- and Chlorocarbons. Acute exposures of rabbits to F-12 result in a dose related depression in left ventricular conduction and cardiac output without changes in heart rate, blood pressure, arterial PO_2, PCO_2, or pH. Intermittant exposure to 10% F-12 does not alter the response to an acute challenge of F-12 suggesting effects are not cumulative. In a study comparing cardiac depression by F-11, F-12, halothane, dichloromethane, had less effect on cardiac output and left ventricular function than the others. As cardiac depressants, F-11 and 1,1,1-trichloroethane appeared as potent as halothane.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of the aerosol "spray can" dispenser has resulted in a very complex and wide variety of materials being available in respirable form. While the acute toxicity of many of the "active ingredients" in such dispensers has been investigated, little is known about the chronic toxicity of these compounds or the toxicity of combinations of these compounds and "inert ingredients" of the pressurized cans. Elucidation of the chronic toxicity of these compounds and their potential combinations can be of vital importance to environmental health.

PROPOSED COURSE: The studies on aluminum are essentially completed. Studies on fluorocarbons will continue with added emphasis on the use of the isolated perfused heart preparation. Commercial products will be investigated on a continuing but infrequent basis.

PUBLICATIONS

Drew, R.T., Gupta, B.N., and Riley, M.T.: Inhalation Studies with Aerosol Oven Cleaners. Presented at the American Industrial Hygiene Association Meeting, 1975 (Abstract).

Drew, R.T., and Taylor, G.J.: Cardiophysiological Studies with Stressed Animals. Proceedings of the 5th Annual Conference on Environmental Toxicology. AMRL-TR-74-125, pp. 121-131, 1974.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80018-06 EBCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical Indices of Inhalation Exposure to Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. T. Drew	Supervisory Physical Scientist	EBCB NIEHS
	C. Harper	Staff Fellow	PB NIEHS
OTHER:	J. R. Fouts	Chief, Pharmacology Branch	PB NIEHS
	J. Patel	Visiting Fellow	EBCB NIEHS
	M. Hogan	Mathematical Statistician	EBB NIEHS
	B. Gupta	Veterinary Medical Officer	EBCB NIEHS
	F. Talley	Supervisory Biological Laboratory Technician	EBCB NIEHS
	J. J. O'Neil	Staff Fellow	EBCB NIEHS
	E. W. Van Stee	Commissioned Officer	EBCB NIEHS
	F. Englebrecht	Visiting Scientist	EBCB NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH
Environmental Biology and Chemistry Branch

SECTION
Inhalation Toxicology Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.7	3.0	3.7

SUMMARY OF WORK (200 words or less - underline keywords)

In this long term project the effects of inhaled solvents are being investigated. Biochemical indices of pulmonary and hepatic injury are being studied using serum enzymes as well as pulmonary and hepatic microsomal enzymes. Oxygen consumption of lung slices is being used to assess toxicity of these agents. Previous compounds investigated include benzene and dichloromethane. Agents presently being studied include xylene, vinyl chloride and vinyl bromide.

PROJECT DESCRIPTION

OBJECTIVES: 1. Determine if pretreatment of animals with compounds which modify hepatic and/or pulmonary microsomal enzymes will alter the acute and chronic toxicity of selected environmental agents including benzene, xylene, vinyl chloride and vinyl bromide.

2. Determine whether inhalation of common solvents effects their enzymatic degradation in lung and liver.

3. Identify the various metabolic pathways involved in the enzymatic degradation of p-xylene and vinyl bromide, and determine whether pretreatment by known inducers stimulates different pathways.

4. Develop systems for assaying the in vitro metabolism of these solvents and investigate variables which alter the rate of metabolism of these compounds.

5. Investigate the uptake oxidation and incorporation of glucose, amino acids and fatty acids by lung tissue and evaluate the relationship between metabolic changes and functional changes occurring after exposure to toxicants, inhibitors, uncouplers or metal ions.

6. Continue the development of the inhalation facility with automated monitoring systems and the capability of handling hazardous materials.

METHODS EMPLOYED: Inhalation exposures are carried out in chambers specifically designed for such studies. They are operated under dynamic conditions of air flow and atmospheric concentrations of specific compounds are monitored at frequent intervals. Biochemical assays are carried out in routine fashion.

MAJOR FINDINGS: Female rats, when treated with p-xylene, showed no change in p-xylene metabolism by hepatic microsomes but a 60% loss in p-xylene metabolizing activity by pulmonary microsomes. The loss in pulmonary xylene metabolizing enzyme activity was observed with three routes of xylene administration (IP, oral and inhalation). Maximum loss of xylene metabolizing enzyme activity was observed two hours after xylene treatment was discontinued. Recovery to normal activity was observed eight days after treatment was discontinued. Phenobarbital increased p-xylene metabolism by hepatic microsomes but did not affect enzyme activity in pulmonary microsomes. Treatment with phenobarbital or antioxidants (vitamin E) did not modify the loss in activity by pulmonary microsomes from xylene-exposed rats.

Male rabbits exposed to 1000 ppm p-xylene for 4 hours showed inactivation of cytochrome P-450 in pulmonary microsomes, but not in hepatic microsomes. This change in P-450 was accompanied by a loss of p-xylene metabolizing activity by rabbit pulmonary microsomes but not rabbit hepatic microsomes. This inactivation occurs when the intermediate, p-tolualdehyde is metabolized

in the absence of aldehyde dehydrogenase in a TPNH requiring reaction. p-Tolualdehyde is synthesized in the liver where the high levels of aldehyde dehydrogenase rapidly convert it to toluic acid. However, small amounts of the p-tolualdehyde are transported to the lung (where there is very little aldehyde dehydrogenase) and cause inactivation of cytochrome P-450.

Rats either untreated or pretreated with phenobarbital were exposed to 20,000 ppm vinyl bromide and exposed for 10 consecutive days. Groups of animals were killed after 5 or 10 exposures and after 5 postexposure days. As with vinyl chloride, phenobarbital and vinyl bromide caused a marked weight depression. Slight, if any, changes were observed in serum enzymes, hepatic P-450 and GSH and normal morphology of liver, lung, kidney, spleen, and heart. Phenobarbital caused a 3- to 4-fold increase in bromide ion concentration in serum over that present in serum as a result of exposure to vinyl bromide only.

Special techniques for using lung tissue slices have been developed. Techniques in tissue handling, slice thickness, shaking rate, gas phase, and method of weight estimation have been varied to determine optimal conditions for estimating metabolism. Current results indicate that amino acids, under certain circumstances, can be oxidized by lung tissue and may account for as much as 50% of the oxygen consumption.

The inhalation chambers have been moved to one central location to enable us to sample all the chambers directly with various detectors, keeping tubing length to a minimum. A device for automatic sampling and gas chromatographic analysis is operational and we are looking into mass spectrographic and IR spectrographic analysis for compounds unsuitable for G.C. analysis.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
In today's society the number of people taking drugs either prescribed by a physician or illicitly is substantial. Some of these compounds stimulate liver microsomal enzymes, and possibly lung systems, thus increasing the rate at which certain chemicals and/or other endogenous substrates are metabolized. These studies are designed to better define the toxic responses to selected solvents and to determine if the toxicity of these solvents can be altered by pre-exposure to themselves or to other drugs. The effect of the route of administration is also being studied.

Benzene was chosen for study because of its widespread industrial and housefold use and because it is now being proposed for use instead of tetraethyl lead in increasing gasoline octane ratings. Paraxylene was chosen because it is also high on the list of widely-used solvents and because little information is known about its toxicity. Vinyl chloride has been investigated because of the recent finding that this compound is a carcinogen. The recent discovery that dichloromethane (a widely-used solvent and degreaser) was metabolized directly to carbon monoxide

by the liver prompted studies with this compound. More recently vinyl bromide was selected because of its similarity to vinyl chloride and because of its use as an intermediate in the manufacture of fire retardants.

Surprisingly little is known regarding pulmonary intermediary metabolism and changes which might occur in response to insult, in spite of the fact that the lung is the first organ exposed to airborne pollutants. It is apparent, that in addition to gas exchange, the lung plays a vital role in the removal, metabolism and release of substances in the blood as well as the synthesis and secretion of substances peculiar to the lung. An understanding of the intermediary metabolism of pulmonary tissue may contribute to our understanding of normal lung function and to changes which occur early in response to toxicant exposure or disease.

PROPOSED COURSE: Solvent toxicity and metabolism and the modification thereof by known inducers and inhibitors of metabolism in liver and other tissues will continue to be investigated. Preliminary results suggest that bromide ion may be responsible for the observed toxicity of vinyl bromide and this hypothesis will be investigated. Other low molecular weight halogenated hydrocarbons will be studied. With regard to intermediary metabolism, we will: (1) evaluate the relative contribution of various substrates (e.g. glucose, amino acids, free fatty acids) to meet the energy requirements of lung tissue; (2) estimate the relative utilization or requirement for various substrates in the presence or absence of other substrates (e.g. difference in glucose utilization in the presence or absence of amino acids); and (3) estimate changes in metabolism produced by metabolic inhibitors and by toxic substances inhaled or administered to animals.

PUBLICATIONS

Drew, R.T., Harper, C., Gupta, B.N., and Talley, F.A.: The Effects of Einyl Chloride Exposure of Rats Pretreated with Phenobarbital. Environ. Hlth. Persp. 11: 235-242, 1975.

Roth, R.P., Drew, R.T., Lo, R.J., and Fouts, J.R.: Dichloromethane Inhalation, Carboxyhemoglobin Levels and Drug Metabolizing Enzymes in Rabbits. Toxicol. Appl. Pharmacol. 33: 427-437, 1975.

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Harper, C., James, M.O., Devereux, T.R., Patel, J.M., Bend, J.R., and Fouts, J.R.: Characteristics and Development of Drug Metabolism by Pulmonary Microsomes. Agents and Action (In Press).

Harper, C., Drew, R.T., and Fouts, J.R.: Benzene and p-Xylene: A Comparison of Inhalation Toxicities and in vitro Hydroxylations. In Active Intermediates: Formation, Toxicity and Inactivation. Ed. R. Snyder. Plenum Publishing Corp., New York, (In Press).

Harper, C.: p-Xylene Metabolism by Rat Pulmonary and Hepatic Microsomes. Fed. Proc. 34, 1975 (Abstract).

Drew, R.T., Patel, J.M., and Van Stee, E.W.: The Effects of Vinyl Bromide Exposure in Rats Pretreated with Phenobarbital or Diethylmaleate. Presented at the Annual Meeting of the Society of Toxicology, March, 1975 (Abstract).

Patel, J.M., Harper, C., and Drew, R.T.: Changes in Serum Enzymes After Inhalation Exposure of p-Xylene in Rats. Presented at the Annual Meeting of the Society of Toxicology, March, 1975.

O'Neil, J.J. and Harper, C.: Alanine Utilization and NH_4 Release by Rat Liver Slices. Physiologist 18: 340, 1975 (Abstract).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
FILE OR NUMBER (DO NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-80020-04 EBCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Changes in Mammalian Pulmonary Function Produced by Inhaled Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. O'Neil	Staff Fellow	EBCB NIEHS
	R. T. Drew	Supervisory Physical Scientist	EBCB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biology and Chemistry Branch

SECTION

Inhalation Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

ABSTRACT OF WORK (200 words or less - underline keywords)

Changes in pulmonary function resulting from inhalation of airborne pollutants are being estimated using a whole body pressure plethysmograph constructed for use with small animals. Both acute and chronic exposure to oxidants, singly and in combination with other compounds result in functional changes. Changes in pulmonary mechanics (e.g. resistance, compliance), spirometry (e.g. lung volumes, frequency of breathing), heart rate. and pulmonary morphology will be estimated.

PROJECT DESCRIPTION

OBJECTIVES: Our objectives are to develop the technology necessary to monitor classical indices of pulmonary function in small animals and to develop more sensitive indicators of pulmonary disease. Changes in pulmonary function caused by inhalation of various environmental agents is of specific interest. These functional changes will be correlated with changes in heart rate and in pulmonary morphology. Compounds of current interest are H_2SO_4 mist, ozone, a combination of the two, aluminum dusts, and an aerosol of the toxin produced by Gymnodinium breve, a dynoflagellate which causes the Florida Red Tide.

METHODS EMPLOYED: A whole body pressure plethysmograph has been constructed for use with laboratory animals. Exposures are conducted with the animal in the plethysmograph so that a control, exposure, and recovery measurement are made with each animal. We can presently estimate changes in pulmonary compliance, resistance, frequency of breathing, minute ventilation, and heart rate.

MAJOR FINDINGS: Plethysmographic equipment has been developed and calibrated and we are now measuring in vivo values for pulmonary compliance and resistance. The equipment is currently being used to study changes in pulmonary function in guinea pigs caused by inhalation of H_2SO_4 mist. Acute exposure to H_2SO_4 mist produces marked increases in pulmonary resistance with a long recovery period necessary before the effects are reversed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Changes in pulmonary function of animals after exposure to inhaled environmental agents are known, in many cases, to be associated with cellular damage and morphologic changes. Development of sensitive methods to detect pulmonary function changes may provide an early marker of lung damage and provide insight into both the mechanism of pulmonary damage and the types of location of the cells most readily injured by specific agents. Correlation of these findings with metabolic and morphologic changes may provide insight into the sequence of pulmonary damage.

PROPOSED COURSE: Future research will focus on three main areas: (1) In order to establish what might be considered normal in vivo values, lung function data will be derived from significant quantities of unexposed animals of various ages. (2) These data will be compared to those from animals in whom functional changes may have occurred either after chronic or during acute exposures to pulmonary irritants. (3) Changes in pulmonary function will be related to any changes in lung morphology or in lung vascular or cardiac functions. Other tests, such as pulmonary diffusing capacity for carbon monoxide will be evaluated.

ENVIRONMENTAL BIOMETRY BRANCH

ENVIRONMENTAL BIOMETRY BRANCH
Summary Statement

The Environmental Biometry Branch fulfills a dual mission, providing statistical, mathematical, data processing, and computer support to the intramural program as well as functioning as an applied research group. The statistical support that is given to the Institute's intramural scientists spans the spectrum of consulting activities from the design of pilot studies to the analysis of data generated at the conclusion of large-scale, long-term experiments. Computer support is equally varied, ranging from the development of information retrieval and inventory maintenance systems to the simulation of complex biological models designed to illucidate mechanistic processes.

The research activities of the Branch cover a diversity of areas including epidemiology, biomathematics, and risk assessment. At the present time, most of the epidemiologic projects are concerned with the statistical analysis of existing data bases in an attempt to detect environmental components and quantify their implications upon human health. Biomathematics, which is essentially a collaborative effort involving a variety of scientists from the Institute's intramural program, focuses mainly on the mathematical modeling of biological processes. Activities in the area of risk assessment range from attempts to broaden the utility of analytical procedures concerned with competing risks to the development of improved statistical techniques for using data from animal carcinogenicity tests conducted at high dose levels to estimate long term risk from chemical carcinogens at very low dose levels.

Statistical Consulting

During the past year the overall volume of statistical support provided to the various branches of the intramural program and to the Office of the Director has increased considerably. Activities in this area ranged from routine processing of small data sets to more extensive collaborative efforts. Statistical support included assessment of study feasibility, assistance with the initial experimental design and appropriate statistical analysis of the data.

The statistical consulting effort involved a wide range of studies. These included experiments investigating the teratogenic and/or toxic effects of compounds such as Dipterex, Captan, dibenzodioxins, and hycanthone analogs. Data from inhalation studies investigating the synergistic potential of ozone and sulphuric acid mist have been analyzed. The comparative sensitivities of different mouse strains and routes of injection were assessed in several studies investigating induction of dominant lethal mutations.

The fit provided by several statistical models to the observed distribution of fetal death in three large groups of control mice was studied. As an outgrowth of a problem involving protein receptor analysis, a generalized non-linear regression algorithm was implemented that allows for weighting of both dependent and independent variables. In a related problem, numerical methods for functional minimization are being investigated. Response surface techniques were employed to estimate optimal combinations of certain parameters to maximize in vitro development of 8-cell mouse embryos.

The Branch has continued to stress the need for its involvement in the initial design stage of the various experiments conducted in the intramural program. While the majority of the statistical support provided to the Institute would still be classified as data analysis, progress is being made in the area of collaboration with respect to experimental design. In a related effort a great deal of attention has been focused on developing self-help systems for routine data analysis available to various laboratory technicians and scientists. Finally, as has been the case in preceding years, data analysis needs continue to generate statistical and mathematical research in the development of new methodology.

Computing

Data processing efforts within the Environmental Biometry Branch fall into two distinct areas - those directed principally to tasks within the Branch, and those dealing primarily with providing data processing services to the Institute at large.

Numerous projects in the latter category have received support. Those of an administrative nature include a computerized warehouse inventory management system, a periodically updated listing of all radioisotopes held at the NIEHS, and for the Budget Office a listing of financial transactions and billings with summaries being provided to each Branch.

In support of intramural research, efforts have been conducted in the fields of protein receptor analysis for the Developmental Toxicology section, chronic study surveillance for the Aerotoxicology section, pharmacokinetic modeling for the Pharmacokinetics section, and gene frequency deviation detection with Monte Carlo simulation of related phenomena for the Population Genetics section. For the Environmental Biology and Chemistry Branch, initiation of an effort which will result in computerized inventories of the Institute's animal population has been effected. The system will provide ready access to large amounts of raw or summarized animal data.

Facilitation of the processing of laboratory data depends on ease of entry of the data to the computer. In this regard, processing of automatically recorded laboratory data has been implemented for several investigators and efforts are continuing to make such capabilities more generally available. The related problem of direct data transfer from laboratory mini-computers to the Environmental Biometry Branch's computers is also being investigated in response to specific needs.

Within the Environmental Biometry Branch, substantial efforts have been directed toward improving the general data processing capability, particularly as it affects the computing needs of those providing statistical data analysis in support of the intramural research program. A DEC GT44 computer has been brought to operational status and has greatly increased the speed and flexibility of communication with the NIH computer center in Bethesda, as well as providing the capability of economically conducting large-scale Monte Carlo simulation studies in diverse areas of statistics and population genetics.

Epidemiology

The major objective of the Epidemiology Program continues to be the identification of various potential health hazards in the general environment. Particular emphasis is given to possible carcinogens, mutagens, and/or teratogens. At the present time, interest is being focused on the carcinogenic and/or reproductive-inhibiting potential of certain organic contaminants of finished drinking water. While some preliminary correlations have been detected, the results often tend to be inconsistent. Therefore, additional analyses are being conducted in an attempt to minimize or eliminate the effects of various confounding factors. In addition, efforts are being made to compare Institute animal data on the postnatal effects of prenatally administered DES with recently published human data in order to quantitatively assess the predictive potential of the animal model developed at the Institute.

Biomathematics

Research is underway in two areas of mathematical biology. Both programs involve collaboration with experimental scientists in other branches.

Joint research with the Environmental Mutagenesis Branch is being conducted on problems in population genetics theory. The long range objective of this work is to develop a better understanding of environmental effects on gene frequency distributions. A model for spatial dispersion of neutral alleles was used in conjunction with drosophila data to indicate the improbability of the same neutral allele appearing at widely separated localities. Some mathematical results needed to study the effect on natural selection of autocorrelated changes in the environment were obtained. A probabilistic relation between the age of a neutral allele and its frequency was derived.

In collaboration with the Pharmacology Branch, research efforts in pharmacokinetics were concentrated in two areas: 1) whole body distribution and modeling studies, 2) uptake, storage, metabolism, and release of pollutants and model compounds by isolated perfused organs, with present emphasis on liver and lung. The kinetics of chemical disposition in whole body and isolated organs are being determined in order to construct mathematical models of these processes which can be used: (a) to better define and understand rate-limiting steps in the process, (b) to extrapolate distribution profiles of chemicals from one tissue to another, one species to another, and to man, and (c) to predict tissue storage of pollutants from knowledge of routes, dose, and numbers of exposures. The disposition of five polychlorinated biphenyls (PCB) isomers was studied in the rat and mouse. Pharmacokinetic models

have been developed for the rat and are presently being developed for the mouse. Disposition data is being obtained for the same compound in the dog and monkey so that species-to-species extrapolation of disposition profiles can be made. Several of these compounds have extremely long half-lives of storage in skin and fat. The pharmacokinetic models will be used to predict accumulation from long term low-level exposure. It has been shown that the lung has a transport system for the removal of prostaglandins. The effect of inhaled environmental pollutants in this system is being investigated.

Risk Assessment for Environmental Carcinogens

Use of data from animal experiments to estimate the human risk from long-term exposure to very low doses of environmental carcinogens poses a number of biological, pharmacological, and statistical problems. One of the statistical problems is to extrapolate the animal dose-response relations from the high dose range where animal test data are available to low doses which humans might encounter. Different techniques which would seem to fit the test data equally well can lead to low dose risk estimates that differ by several orders of magnitude. The purpose of this project is to evaluate existing methods and to develop new statistical methods for risk estimation and animal test design, reflecting current understanding of carcinogenic mechanisms.

The low-dose extrapolation properties of several existing models such as the probit model and the one-hit model were studied along with different methods of accounting for background incidence and induction time. A number of different modeling approaches consistent with biological principles suggest that cancer incidence should be roughly proportional to dose in the low-dose range; this is in contrast to the probit model, which assumes that incidence decreases much more rapidly with decreasing dose in the low-dose range. It was shown theoretically that cancer induction time would have to be strongly dose-dependent in order to have much effect on low-dose risk estimates. A new technique for low-dose extrapolation has been developed mathematically and incorporated into computer programs. "Best estimates" of increased risk over background are calculated along with confidence intervals for the estimates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 40002-06 EBB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Statistical Methodology Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. K. Haseman	Mathematical Statistician	EBB NIEHS
	F. O. Lin	Visiting Fellow	EBB NIEHS
	B. Singh	Visiting Fellow	EBB NIEHS
OTHER:	E. R. Soares	Senior Staff Fellow	EMB NIEHS

COOPERATING UNITS (if any)

Environmental Mutagenesis Branch

LAB/BRANCH
Environmental Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct research on statistical methodology problems related to the Branch's consulting activities. These efforts include the development of new statistical tests and the modification of existing procedures to meet specific Institute needs. In this research effort, particular emphasis has been placed on nonparametric statistical methods.

PROJECT DESCRIPTION

OBJECTIVES: To conduct statistical research on various problems related to the Branch's consulting activities.

METHODS EMPLOYED: Statistical methodology ranging from Monte-Carlo simulation techniques to the theoretical modification of existing test procedures has been utilized to produce analytical techniques applicable to problems arising in various intramural laboratory studies.

MAJOR FINDINGS:

(1) A previously published nonparametric test for interaction could be adversely affected by the presence of first-order effects in the data. A modification of the test was developed that mitigated this difficulty.

(2) Neither the commonly employed Poisson nor binomial models for fetal death were found to adequately explain the distribution of fetal death in three large sets of mouse control data. Certain statistical tests based on these inappropriate models were shown to seriously exaggerate significance levels. Until a satisfactory parametric model for fetal death is derived, nonparametric test procedures are recommended.

(3) Jonckheere's nonparametric test for dose-response was modified to allow for ties at a single extreme value. Exact small sample tables of the modified test statistic were calculated, and a large sample approximation was derived.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

(1) The expansion of statistical methodology for the evaluation of interaction, particularly in a nonparametric framework, is directly related to the Institute's growing concern with the potential synergistic effects of environmental compounds.

(2) The accurate assessments of variations in the distribution of fetal deaths among different treatment groups is essential in the performance of dominant lethal studies.

(3) Since many of the treated as well as most of all of the control liters may have no affected fetuses in the teratological investigations of rare anomalies, the modification of Jonckheere's test described above is of significance to the field of teratology.

PROPOSED COURSE: To continue statistical methodology development with particular emphasis on nonparametric multiple comparison procedures.

PUBLICATIONS

Haseman, J. K. and Hogan, M. D.: Selection of the experimental unit in teratology studies. Teratology 12: 165-171, 1975.

Lin, F. O. and Haseman, J. K.: A modified Jonckheere test against ordered alternatives when ties are present at a single extreme value. Biometrische Zeitschrift. (In press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 41001-02 EBB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Risk Assessment for Environmental Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. G. Hoel	Chief	EBB NIEHS
	H. A. Guess	Staff Fellow	EBB NIEHS
OTHER:	K. S. Crump	Visiting Scientist	EBB NIEHS
	C. H. Langley	Senior Staff Fellow	EMB NIEHS
	P. Y. Chi	Visiting Fellow	EBB NIEHS

COOPERATING UNITS (if any)

Environmental Mutagenesis Branch

LAB/BRANCH
Environmental Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop improved statistical techniques for using data from animal carcinogenicity tests conducted at high dose levels to estimate long term risk from chemical carcinogens at very low dose levels. Existing techniques are evaluated and new techniques are studied, with particular emphasis on those which attempt to relate mechanistically to the carcinogenic process.

PROJECT DESCRIPTION

OBJECTIVES: In order to estimate human risk based on animal carcinogenesis experiments, it is currently necessary to extrapolate data from high dose experiments to low doses. The purpose of this project is to study the various mathematical and probabilistic methods for extrapolation with particular emphasis on those which attempt to relate mechanistically to the carcinogenic process.

METHODS EMPLOYED: Various possible models of carcinogenesis were analyzed with respect to low dose kinetics. Considerations of mechanism such as hit models and multistage models were compared to so-called log normal response models. The importance of background incidence and induction time were evaluated. These considerations led to the investigation of "best" risk estimates and optimal experimental designs.

MAJOR FINDINGS: 1) If carcinogenesis by an external agent is considered to be augmenting an already on-going spontaneous process, then the response will be linear at low dose. 2) If the origin of a cancer is an event in a single cell (as distinct from a whole tissue) then the low dose response will be linear. 3) In general, time of cancer induction must be heavily dependent on dose rate for such consideration to play a role in low dose risk. 4) Linear extrapolation may not always be the conservative technique it has been thought to be. 5) The optimal experimental dose for obtaining the maximal acceptable dose is robust with respect to models when one is extrapolating linearly. 6) A statistical technique for low-dose extrapolation of dichotomous data has been developed mathematically and programmed. The technique is based on a general multistage model, where the number of hits is treated as an unknown to be estimated along with the dose-response coefficients. Best estimates of increased response above background are calculated along with confidence intervals for the estimates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project potentially will have an impact upon human risk assessment of environmental carcinogens. This in turn should affect the regulatory process. Further, the techniques developed in this project may be applicable to toxicological effects other than carcinogenesis.

PROPOSED COURSE: 1) Modify the hit models to allow a hit cell to be repaired by some stochastic mechanism and determine if repair can have any significant qualitative effect upon the dose response curve. 2) Consider the implication of variance among individuals in sensitivities to an insult of a carcinogen upon low dose risk estimation. 3) Conduct simulation studies to check the theoretical confidence intervals and goodness-of-fit of the "best" estimates. 4) Study models to estimate time-to-occurrence. 5) Evaluate existing carcinogenesis data for species variabilities. 6) Apply best estimate techniques and existing conservative extrapolation methods to various data sets for comparative purposes.

PUBLICATIONS

Chand, N. and Hoel, D. G.: A comparison of models for determining safe levels of environmental agents. In proschan, Frank and Serfling, R. J. (Eds.): Reliability and Biometry. Philadelphia, SIAM, 1974, pp. 681-700.

Crump, K. S., Hoel, D. G., Langley, C. H., and Peto, R.: Fundamental carcinogenic processes and their implications for low dose risk assessment. Cancer Res. (in press)

Guess, H. A. and Crump, K. S.: Low-dose extrapolation of data from animal carcinogenicity tests - analysis of a new statistical technique. Mathematical Biosciences (in press)

Hoel, D. G.: Statistical models for estimating carcinogenic risks from animal data. In Proceedings of the Fifth Annual Conference on Environmental Toxicology. AMRL-TR-74-125 Washington, D. C., U.S. Government Printing Office, 1974, 7 pp.

Hoel, D. G.: Statistical extrapolation methods for estimating risks from animal data. NYAS Conference on Occupational Carcinogenesis. Proc. Annals of the New York Academy of Sciences (in press)

Hoel, D. G., Gaylor, D. W., Kirschstein, R. L., Saffiotti, U. Schneiderman, M. A. Estimation of risks of irreversible, delayed toxicity. J. Tox. & Environ. Health 1: 133-151, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 41002-01 EBB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Statistical Estimation of Specific Risks in Disease Incidence Data

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. J. Mitchell	Visiting Scientist	EBB NIEHS
	D. G. Hoel	Chief	EBB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biometry Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The statistical analysis of censored disease incidence data in groups of animals subjected to a treatment depends on the designation of the disease as "lethal" or "non-lethal", the analysis being different for the two cases. In this research, a unified method is developed which makes use of information derived from incidental observations of the disease as well as deaths due to the disease. In particular, an extension of the Kaplan-Meier method is given for graphically summarizing the results for a single group. The same approach will be used to develop methods for comparing a treated group with a control, and, in particular, the estimation of relative risks for both the time to incidence of and the time to death from the disease.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To develop new statistical methods for the analysis of disease incidence data. In particular, to develop methods that utilize information derived from "incidental" deaths as well as deaths directly attributable to the disease.

METHODS EMPLOYED: The Kaplan-Meier method is a well-known technique for estimating the distribution of times to death from a specified disease, corrected for competing risks (deaths due to other causes). With this method, cases in which the disease is present at death but is not the cause of death are treated as if the disease were not present. This research is concerned with extending the Kaplan-Meier approach to utilize information from these "incidental" observations of the disease. This will lead to new procedures for comparing a treated group with a control group, and, in particular, to estimates of relative risks for both the time to incidence of the disease and the time to death from the disease.

MAJOR FINDINGS: A computer program has been written to estimate the distributions of times to incidence and times to death from a specified disease, using information from incidental observations of the disease as well as deaths from the disease.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This research will have application to the analysis of disease incidence data from animal experiments, clinical trials, or epidemiological studies. It extends and unifies present statistical procedures in the area of competing risks, and removes the necessity to analyze separately non-lethal and lethal occurrences of the disease.

PROPOSED COURSE: Work will continue to utilize this approach on the estimation of various measures of relative risk and confidence intervals for these measures.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 42002-04 EBB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Disposition, Pharmacokinetic Models, and Species-to-Species Extrapolation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Anderson	Senior Staff Fellow	EBB NIEHS
	T. E. Eling	Research Chemist	PB NIEHS
	H. B. Matthews	Research Chemist	PB NIEHS
	D. B. Tuey	NIH Postdoctoral Fellow	EBB NIEHS
OTHER:	R. L. Dedrick	Section Chief	BEIB DRS
	R. J. Lutz	Chemical Engineer	BEIB DRS

COOPERATING UNITS (if any)

Chemical Engineering Section, BEIB, DRS, NIH

LAB/BRANCH
Environmental Biometry Branch and Pharmacology Branch

SECTION
Pharmacokinetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to understand the mechanisms which determine the disposition of environmental chemicals in various animal species and to develop pharmacokinetic models for these chemicals. After developing pharmacokinetic models in several species, it may be possible to extrapolate disposition of chemicals to other species, and, ultimately, to man. The prediction of the accumulation in man after low-level chronic exposure could then be done. Pharmacokinetic information (models) can be very useful in the design and interpretation of toxicity studies. The topics of present interest are: 1) Disposition of several polychlorinated byphenyl isomers in the rat, mouse, dog, and monkey. 2) Development of pharmacokinetic models in these species.

PHS-6040
(1-8-75)

PROJECT DESCRIPTION

OBJECTIVES: The basic objective is to understand the mechanisms which contro the distribution, storage, metabolism, and excretion of lipophilic chemicals, such as polychlorinated biphenyls (PCB), in various species and to extrapolate this information from species to species.

METHODS EMPLOYED: The approach used by R. L. Dedrick and D. L. Zaharko in the development of pharmacokinetic models for several drugs is being employed to develope similar type models for environmental pollutants.

MAJOR FINDINGS: Pharmacokinetic models have been developed for PCB, 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro- and 2,4,5,2',4',5'-hexachlorobiphenyl, in the rat. These models can predict the disposition up to 42 days after a single i.v. dose. The biochemical and physiological parameters used in this model were determined from studies in bile-cannulated animals, isolated perfused liver studies, and disposition studies following a single i.v. dose. The adipose to blood partitioning coefficient for the hexachlorobiphenyl that we observed in rat was similar to that reported for humans. Values for the metabolic kinetic parameters are the most difficult to obtain, and we have the least confidence in these parameters. Additional experiments are being planned which will enable us to determine better estimates for these parameters.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many environmental pollutants such as PCBs have very long environmental half-lives. Man can be exposed to these compounds for long periods of time. After developing pharmacokinetic models in several species, an extrapolation of the disposition of these compounds to man may be possible. The prediction of the accumulation in man after low-level chronic exposure could then be done. Pharmacokinetic information (models) can be very useful in the design and interpretation of toxicity studies. In particular, toxicity comparisons between species should be accompanied by disposition comparisons and extrapolation of toxicity results obtained in experimental animals to man should be accompanied by extrapolations of disposition studies. Knowledge of the ability of xenobiotics to cross blood-tissue barriers such as the blood-testicular and blood-placental barrier is required by the teratology and mutagenesis groups at the Institute. Although detailed pharmacokinetic models may not be necessary in each case, species to species extrapolation of transfer rates (blood to tissue) are necessary.

PROPOSED COURSE:

1) Pharmacokinetic models for the four PCBs studied will be developed in the mouse from the data we have obtained in our laboratory.

2) Disposition studies of these PCBs will be done in rat and mouse after multiple doses. The ability of the models to predict disposition in these species after multiple doses will be tested.

3) Data for the disposition of these PCBs in monkey and dog will be obtained through a contract. This data will be used to construct models in these species.

4) Pharmacokinetic models will be developed for two additional PCBs, 3,5,3',5'-tetrachlorobiphenyl and 2,3,6,2',3',6'-hexachlorobiphenyl in rat and mouse from the data obtained in our laboratory.

5) From a comparison of the parameters used in the models for the various species, we want to extrapolate to values of these parameters for man. Then a pharmacokinetic model for man can be constructed. Predictions of accumulation in man after chronic low-level exposure will be made.

6) Attempts will be made to obtain disposition data for xenobiotics in man. We are currently involved in two projects which will hopefully give us some information, these being studies on the pharmacokinetics and toxicity of Kepone in humans and similar studies with the polybrominated biphenyls.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 43001-04 EBB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Epidemiological Investigations of Potential Human Health Hazards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. D. Hogan	Mathematical Statistician	EBB NIEHS
OTHER:	P. Y. Chi	Visiting Fellow	EBB NIEHS
	B. Singh	Visiting Fellow	EBB NIEHS

COOPERATING UNITS (if any)

Environmental Toxicology Branch, UNC School of Public Health, and State Department of Human Resources

LAB/BRANCH
Environmental Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to identify and/or confirm the presence of various potential health hazards in the general environment, with particular emphasis being given to possible carcinogens, mutagens, and teratogens. At the present time interest is being focused on the carcinogenic and/or reproductive-inhibiting potential of certain organic contaminants of finished drinking water. Comparisons of animal and human data with respect to the postnatal effects of prenatally administered DES are also being initiated.

PROJECT DESCRIPTION

OBJECTIVES: To enumerate and assess the significance of various environmental factors in the etiology of carcinogenic, teratogenic, mutagenic diseases. Particular emphasis is given to the extention of intramural laboratory findings to the human setting fot the dual purpose of (a) identifying (possibly at a preclinical stage) potential high-risk subgroups in the general population and (b) developing realistic animal models for prodicting human response.

METHODS EMPLOYED: A variety of statistical procedures (usually non-parametric) has been and is being employed to evaluate existing data bases in terms of their environmental health significance.

MAJOR FINDINGS:

(1) Initial analyses of data from two separate studies of the potential health effects of organic contamination of finished drinking water suggest that

(a) While there were a few instances of positive association between levels of specific contaminants and 20-year, county-wide, age-adjusted cancer incidence rates ofr various sites, the results did not tend to be consistent across sexes, races, and/or separate study populations; and

(b) Chloroform levels may be inversely associated with one of the measures of fertility that is being examined among cities of high population density.

(2) A reanalysis of some of the published human data relating to the potential health hazards incurred by the offspring of mothers exposed prenatally to DES indicates that (a) the frequently quoted figure of 4 per 1000 as the estimated upperbound on the risk of developing vaginal adenocarcinoma could by an under estimate, and that (b) the 4 cases of squamous-cell carcinoma in situ of the cervix observed in the Mayo Clinic study may be more significant than was indicated by the original investigators.

PROPOSED COURSE:

(1) The analyses of the water data will be completed as soon as possible, controlling for all confounding factors so as to reduce, if not totally eliminate, the contradictions existing in the preliminary results. When the findings relating to the reproductivity phase of the analysis are completed, they should be compared to ongoing intramural research in the same area.

(2) Site-specific cancer trends over at least the last 10 years will be examined for both the nation and state to see if they can be related in any way to changes in patterns of environmental exposure.

(3) An attempt will be made to correlate Institute animal data on DES exposure with corresponding published human data in order to quantitatively assess the animal model.

(4) A collaborative study of adverse outcomes of pregnancy and potential etiological factors with Dr. Craig Turnbull of the UNC School of Public Health has been initiated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 44002-01 EBB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mathematical Population Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Guess	Staff Fellow	EBB NIEHS
	J. H. Gillespie	Senior Staff Fellow	EMB NIEHS
	C. H. Langley	Senior Staff Fellow	EMB NIEHS
	G. A. Watterson	Visiting Scientist	EBB NIEHS
	K. S. Crump	Visiting Scientist	EBB NIEHS

COOPERATING UNITS (if any)

Environmental Mutagenesis Branch

LAB/BRANCH
Environmental Biometry Branch

SECTION

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to develop a better understanding of environmental effects on gene frequency distributions. The work is conducted in collaboration with scientists in the Environmental Mutagenesis Branch. Current research topics are: (1) spatial dispersion of neutral alleles; (2) the relation between the frequency of a neutral allele and its age; (3) effects of autocorrelated random environments on the progress of natural selection.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To work jointly with scientists in EMB on problems in population genetics theory. This work, along with experimental population genetics studies conducted by EMB, is aimed at developing a better understanding of environmental effects on gene frequency distributions.

MAJOR FINDINGS: A model for spatial dispersion of neutral alleles was used in conjunction with drosophila data to indicate the improbability of the same neutral allele occurring at widely separated localities. Mathematical results needed to study autocorrelated (nonindependent) environmental effects were derived. A well-known problem in the population genetics literature, regarding the theoretical relation between the age of a neutral allele and its frequency, was solved. Results of current research in theoretical population genetics related to the Institute's program were reviewed at a conference sponsored by EMB and EBB.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of environmental effects on gene frequency distributions contributes to a knowledge of how genetic variability should be taken into account in predicting long-term effects of environment changes on public health.

PROPOSED COURSE: Research on effects of autocorrelated random environments will continue. Somewhat less effort is projected since the scientists with whom the joint work was done have left the Institute.

PUBLICATIONS

Crump, K. S. and Gillespie, J. H.: The dispersion of a neutral allele considered as a branching process. J. Appl. Prob. (in press)

ENVIRONMENTAL BIOPHYSICS BRANCH

ENVIRONMENTAL BIOPHYSICS BRANCH
Summary Statement

The research programs of the Environmental Biophysics Branch are focused primarily in two areas - Nonionizing radiation (microwaves) and Noise effects. Additional efforts concern especially respiratory tract deposition models and dose-response relationships with ionizing radiation and radioactive elements.

Nonionizing radiation:

Some objectives of the program in microwaves are: (1) to develop additional technics for microwave exposure to continuous wave, pulsed wave, and modulated wave delivery modes and known field characteristics; (2) to develop dosimetric technics (both instrumental and analytical); (3) to design and build a system for evaluating electrical properties of biological material; (4) to study biophysical mechanisms of interaction of continuous wave, pulsed and modulated microwave radiation (of frequencies from 1 to 10 GHz) with biological samples at cellular and macromolecular levels; (5) to evaluate the effects of continuous wave and pulsed 2.45 GHz microwave radiation on embryonic development and reproductive functions of exposed animals; (6) to study the effects of continuous wave and pulsed 2.45 GHz microwave radiation on the development of the immunological response in animals; and (7) to investigate the effects of continuous wave, pulsed and modulated microwave radiation at frequencies of 1 to 10 GHz on central nervous system functions and behavior in animals.

Some highlights of recent research in this program include: (1) We are now able to expose biological specimens to various kinds of microwave radiation differing in wave character as well as frequency. We can also accurately determine the radiation dose (incident as well as absorbed) in biological specimens. The exposure area is located within environmental chambers so as to study the non-thermal effects of microwave radiation.

Experiments on cells have included studies on effects of microwave radiation on rabbit erythrocytes and lymphocytes and comparison of results with those reported by Polish scientists. The studies on lymphocytes is part of an overall investigation of the effects of microwaves on immunological responses.

Studies have been made of microwave effects on fertilized Japanese quail eggs and on the reproductive activity of quail from the exposed eggs. Organs from quail exposed in ovo and allowed to mature were also studied. These studies together with others using pregnant mice will give some idea of potential danger of microwaves for pregnant animals and man. Result of these studies need further confirmation and extension, but suggest a variety of effects on fertilized quail eggs including decreased fertility in quail from exposed eggs and abnormalities in liver, bursa and spleen of quail from exposed eggs.

Studies on microwave effects on nervous tissue include exposure of spinal cords of cats to microwaves and measurements of synaptic transmission and reflex responses.

Studies on contract have involved effects of microwave, electric field and magnetic field exposure on biological rhythms in rats, conditioned behavior in primates, and urinary concentrations of metabolites and indicators of neuro-hormone secretions. These latter studies may help answer questions about possible health effects of radiation from high voltage lines.

Effects of nonionizing radiation on nervous function and behavior are of special interest since the USSR has established much lower levels of radiation as safety standards than have we (USA) based primarily on effects of microwave radiation on CNS and behavior. We need to know more about the effects of nonionizing radiation on nervous functions before we can interpret the Russian experiments and arrive at any estimate of health hazards.

Experiments to determine the long-term chronic effects of exposure to microwaves are especially needed. The Soviet Union standards are based primarily on behavioral effects of long-term chronic exposures to microwaves. During the last year we have solicited contract proposals to perform chronic microwave exposure in animals. This work will begin soon.

Noise effects:

The efforts of this group are more or less equally divided between (1) programs in developing new methods to deliver and measure sound and signals within the mammalian auditory chain - by - direct activation of the ossicular chain and probes capable of recording sounds at the inner ear-basilar membrane, and (2) effects of chemicals and drugs on basic mechanisms of hearing.

In terms of the latter program, studies were made on the effect of methyl mercury on nerve action potentials of the inner ear and on concentrations of methyl mercury in the inner ear fluids.

Ototoxic antibiotics and noise were studied separately and in combination for their effect on the function and metabolism of hair cells in the inner ear. Effects of noise and ototoxic chemicals on ion flux in the perilymphatic space are also being studied as part of this program. The basic objectives are to define the sites of action of chemicals in combination with noise and to understand why synergisms of effect may occur.

Other:

Contracts and workshops on respiratory tract deposition of particles and dose response relationships with ionizing radiation effects are described in some detail in the specific annual reports. The respiratory tract deposition studies are of interest to other groups (i.e., Aerotoxicology) whose programs are especially concerned with factors affecting, lung functions and the possible role of particles in carrying chemicals toxic to lung. The dose-response relationships conference is of interest to the general NIEHS program in extrapolations where the major considerations are of how one extrapolates from high

dose, acute effect to low dose-chronic effects. The best dose-response relationship data seems to be in ionizing radiation effects, and the question is whether this is relevant to chemical-induced injury.

Personnel:

Activites of Branch personnel have been numerous and varied. Many functions are interagency involving things like opinions on criteria documents (noise and microwaves especially, but also with regard to ionizing radiation), review of contracts and grant proposals to other agencies, and various committees. The following is an attempt to be comprehensive:

1. Reginald Cook: member, Interagency Noise Effects Panel; member, Committee on Hearing, Bioacoustics and Biomechanics of NRC; invited lecturer, seminars on effects of noise - courses on speech, hearing and acoustical engineering, UNC and NCSU.

2. Dr. Phillip E. Hamrick: NIEHS Radiation Safety Officer

3. Dr. Donald I. McRee: Adjunct Assistant Professor, NCSU; seminars - Department of Engineering Science and Mathematics, NCSU; Coordinator, US-USSR Cooperative Program on Health Effects of Non-ionizing Radiation; NIEHS representative on Inter-departmental Radiation Advisory Committee (IRAC) on Biological Effects of Non-ionizing Radiation; Chairman of Scientific Information Exchange Working Group; representative for DHEW on Interagency Advisory Committee on Electric Field Effects from High Voltage Transmission Lines (organized by ERDA); representative on American-National Standards Institute C-95 Committee on Safety Standards of Non-ionizing Radiation; wrote book chapter for Annual Review of Medicine; invited paper on embryological effects of microwaves, Electromagnetic Radiation Advisory Council, Office of Telecommunications Policy.

4. Dr. Teruzo Konishi: invited lecturer, 20th Audiology Society Meeting, Osaka, Japan; seminar at University of Pittsburgh Medical School; member, Technical Committee, Physiophysiological Section, Acoustical Society of America.

5. Dr. Phillip J. Walsh: Adjunct Appointment, UNC - graduate committees and lectures to graduate students; member of Ph.D. Candidate Committees and collaboration with nuclear physics program at NCSU; member, Nuclear Subpanel, Committee on Materials, Federal Council on Science and Technology; member, Federal/State Advisory Panel on Uranium Mill Tailings Remedial Action Program; conducted interagency workshop on Theoretical Dose-Response Relationships; chairman, NIEHS Radiation Safety Committee; member, NIEHS Library Committee.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50002-03 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Ototoxicity of Methyl Mercury

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	EBpB	NIEHS
Others:	Philip E. Hamrick	Senior Staff Fellow	EBpB	NIEHS
	Phillip J. Walsh	Radiation Physicist	EBpB	NIEHS
	Alexandra Yankwich	Health Services Officer	EBpB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the relationship between the distribution of methyl mercury in the cochlear fluids and the alteration of the cochlear potentials in guinea pigs. Animals are treated with methyl mercury chloride labeled with ^{14}C and/or ^{203}Hg. Radioactivity in the endolymph, perilymph, cerebrospinal fluid and whole blood is measured with a liquid scintillation counter. The stimulus-related responses as well as the DC polarization of the endolymph are recorded from the cochlea. The cochlear microphonic and action potential are suppressed by methyl mercury but the endolymphatic potential remains unchanged. No specific accumulation of methyl mercury is found in either the perilymph or endolymph.

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to study the effect of methyl mercury on cochlear potentials and to determine the concentration of methyl mercury in the cochlear fluids.

METHODS EMPLOYED: One group of guinea pigs was treated with a daily subcutaneous dose of methyl mercury chloride labeled with ^{14}C, with a total dose of 17 mg/gm of body weight for a period of seven days. In two other groups of guinea pigs, methyl mercury chloride with ^{203}Hg was given intravenously with total doses of 0.2 mg/kg and 17 mg/kg of body weight, respectively. The cochlear potentials were measured in the basal and third turns with differential electrodes. Methyl mercury concentrations in the endolymph, perilymph, cerebrospinal fluid and whole blood were determined with a liquid scintillation counter.

MAJOR FINDINGS: When animals received subcutaneous administration of 17 mg/kg (2.4 mg/kg per day) of methyl mercury chloride labeled with ^{14}C each day for a period of one week and were tested one day after the last treatment, the action potential in response to 6 and 8 KHz showed suppression. The cochlear microphonics were suppressed more markedly in the basal turn than in the third turn of the cochlea. The endocochlear potential was found to be in the normal range. The concentration of methyl mercury in the cochlear fluids was about 0.4% of the concentration of methyl mercury in the whole blood.

When 0.2 mg/kg of $CH_3{}^{203}HgCl$ was injected intravenously, results showed that the methyl mercury level in whole blood dropped rapidly during the first day after injection. After this initial fast decrease, the blood level fell more slowly with a half time of about one week. The concentration of methyl mercury in the perilymph, endolymph and CSF rose during the first day. Their peaks appeared at the end of the first day. The initial decline was rapid and the concentration of methyl mercury in cochlear fluids and CSF appeared to fall slowly with a half time similar to that of the blood.

When doses of the intravenous injection were increased to 17 mg/kg, the kinetics of methyl mercury uptake were similar to that observed with the lower dose of methyl mercury. However, the initial fast decline in the blood level was slower and peaks in the cochlear fluids and CSF appeared within 24 hours. The concentration after 10 days was 10^{-2} μgm/gm in the cochlear fluids and CSF.

A double labeled experiment with $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ was carried out. The ratio of ^{203}Hg to ^{14}C was 0.5. The blood and CSF showed 0.44 and 0.48, respectively after seven days indicating that most of the mercury detected was in the form of methyl mercury. The concentration of methyl mercury in the cochlear fluids did not show specific accumulation in either the perilymph or endolymph.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Clinical reports of methyl mercury poisoning shows sensorineural hearing loss. The knowledge obtained by this project is important for an understanding of the ototoxic effect of methyl mercury.

PROPOSED COURSE: The work will be continued to collect more data as time permits. In addition, the permeability of the cochlear partition and the ionic concentration of the cochlear fluids will be examined in methyl mercury-treated guinea pigs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50003-03 EBpB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physiological Study of Auditory Fatigue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	EBpB	NIEHS
Others:	Reginald Cook	Sanitary Engineer	EBpB	NIEHS
	Alexandra Yankwich	Health Ser. Officer	EBpB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Biophysics Branch

SECTION

None

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

The long-term objective of this project is to explore the location and nature of physiological changes underlying short-term post stimulatory suppression. The present project is designed to study the relationship between the endocochlear potential and the cochlear microphonics during auditory fatigue in guinea pigs.

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to study the effect of auditory fatigue on the endocochlear potential and to explore the nature of physiological changes underlying the temporal threshold shift.

METHODS EMPLOYED: The cochlear microphonic (CM), action potential (AP) and endocochlear potential (EP) were recorded in anesthetized guinea pigs. The temporary suppression of EP produced by a short-term exposure tone, and the recovery process of CM and AP were examined.

MAJOR FINDINGS: When an exposure tone of high frequency was delivered for two minutes, EP remained unchanged until CM reached the limit of linearity of its input-output function. EP recorded from the basal turn of the cochlea decreased its magnitude when the sound pressure level exceeded the limit of linearity of the input-output curve of CM. EP shift increased its magnitude by further increase of sound level of exposure tone. In addition, both EP shift and CM showed temporary fatigue during the exposure. EP returned to the pre-exposure level when the sound pressure level did not exceed the level which produced the maximum amplitude of CM. Further increase of sound intensity resulted in overshoot of EP during recovery after exposure. Frequently this was associated with the positive summating potential.

When EP was recorded from the basal and third turns of the cochlea and frequency of the exposure tone was 6 to 8 KHz, EP in the third turn remained unchanged, whereas EP in the basal turn showed a negative shift during exposure. When the perilymphatic space was perfused with K-rich solution (K:50 mM), CM was greatly reduced but EP increased its magnitude by 5 to 10 mV. When a high-intensity exposure tone was delivered during perfusion, no EP shift was observed. When guinea pigs were continuously exposed to octave band noise (CF, 2 KHz) of 110 dB SPL over a period of 10 days, the input-output curve of CM was shifted downward by more than 6 dB; the amplitude of AP was also reduced and rapid growth of AP during a test tone of low intensity could not be observed; EP in the basal turn of the cochlea was found to be in the normal range. EP shift produced by a high intensity exposure tone of 6 KHz was smaller than that observed in controls not pre-exposed to loud noise.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The ability of environmental noise to produce hearing loss over a long period of time can be predicted from the temporary threshold shift. The knowledge obtained from this project is important for an understanding of the physiological process upon which the temporary threshold shift depends.

PROPOSED COURSE: The auditory fatigue and the recovery process of the single auditory nerve fiber will be measured in cats or guinea pigs. We are planning to use a laboratory computer to generate pseudo-random noise as a stimulus and to measure the cross-correlation between single unit response and acoustic stimuli.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50005-03 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Noise and Ototoxic Agents on Energy Balance and Metabolism in Cochlea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	EBpB	NIEHS
Others:	Phillip J. Walsh	Radiation Physicist	EBpB	NIEHS
	Philip E. Hamrick	Senior Staff Fellow	EBpB	NIEHS
	Alexandra Yankwich	Health Ser. Officer	EBpB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to increase the understanding of selected basic auditory mechanisms under normal conditions and under the influence of sensory disorder and physical/chemical agents. The present topics are: (1) permeability of cochlear partitions to sodium and potassium ions, and (2) effect of locally applied ototoxic agents on cochlear potentials.

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to study changes in the permeability of cochlear partitions to various ions and ototoxic drugs and to reveal specific mechanisms of noise- or drug-induced hearing loss.

METHODS EMPLOYED: Stimulus-related cochlear potentials were recorded and the endocochlear potential was monitored in anesthetized guinea pigs. Using microperfusion techniques, endolymph or perilymph was replaced with a solution containing various concentrations of neomycin, kanamycin and dihydrostreptomycin. Cochlear potentials were recorded before, during and after perfusion. The permeability of the cochlear partition to ^{22}Na and ^{42}K was determined by perfusing the perilymphatic space with artificial perilymph containing ^{22}Na and ^{42}K. Samples of endolymph, perilymph in scala vestibuli, and perilymph in scala tympani were collected. Concentrations of ^{22}Na and ^{42}K in these samples were determined using a gamma-spectrometry system incorporating a germanium detector, multichannel pulse height analyzer and associated electronic components.

MAJOR FINDINGS: Sulfate compounds of dihydrostreptomycin, kanamycin and neomycin were used. The concentrations of these antibiotics varied from 5×10^{-3} to 10^{-6} g/ml. Cochlear microphonics and action potentials were more markedly suppressed by application into the endolymph than into the perilymph. Perilymphatic administration of either dihydrostreptomycin or neomycin did not produce any appreciable effect on the cochlear microphonics, action potentials or endocochlear potentials. In contrast, endolymphatic application of neomycin of 10^{-4} g/ml concentration resulted in a marked reduction of sound-evoked responses and endocochlear potentials. Recovery was usually incomplete once decreases of the cochlear potentials were observed during the administration.

When the perilymphatic space was continuously perfused with artificial perilymph containing ^{22}Na and ^{42}K at the rate of 2 µl/min over a period ranging from 10 to 40 minutes, the electrical responses of the cochlea remained unchanged. Concentrations of ^{22}Na and ^{42}K in the perilymph ranged from 60 to 90% of those in the perfusate. Concentrations of ^{42}K in the endolymph were higher than those in the perfusate, increasing with the duration of the perfusion. Concentrations of ^{42}K in the endolymph were 197, 247 and 377% of perfusate concentrations when the duration of perfusion was 10, 20 and 40 minutes respectively. Concentrations of ^{22}Na in the endolymph were less than 2% of perfusate concentrations. In post-mortem states, the uptake of ^{42}K was small. Concentrations of ^{22}Na in the endolymph increased.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The permeability of the cochlear partition to various ions or ototoxic antibiotics has been frequently hypothetized but supporting data are rather limited. Present results seem to be of value in pinpointing sites of action of antibiotics on hair-bearing ends of hair cells. The permeability of the cochlear partition to ^{22}Na and ^{42}K clearly indicates that high concentrations of K and low concentrations of Na in the endolymph are maintained by active ion transport.

PROPOSED COURSE: The permeability of the cochlear partition to other ototoxic agents will be studied. In addition, tritiated antibiotics will be used to determine the distribution of antibiotics in the cochlear fluids. The permeability of the cochlear partition to Na and K ions will be studied further not only in normal controls, but also in animals exposed to noise, microwaves, oxygen deprivation, low temperature, or treatment with metabolic inhibitors, ATPase inhibitors and ototoxic drugs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50006-02 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Responses of Listeners to Speech Recovered from the Inner Ears of Guinea Pigs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Sanitary Engineer	EBpB	NIEHS
	Teruzo Konishi	Visiting Scientist	EBpB	NIEHS
	Charles Hamm	Physical Science Technician	EBpB	NIEHS
Other:	W.G. Thomas	Director, Speech and Hearing Clinic	NCMH	UNC

COOPERATING UNITS (if any)

Speech and Hearing Clinic, N.C. Memorial Hospital, University of North Carolina

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.15	.25

SUMMARY OF WORK (200 words or less - underline keywords)

This project is an extension of Project Z01 ES 50006-01 in which signals were imparted into the ossicular chain by normal acoustic methods and by direct activation by piezoelectric type transducers, and the resulting inner ear responses electrophysiologically recovered and subsequently presented to listener panels in discrimination tests. The current project was designed so that listener panels could compare electrophysiologically recovered speech initiated by (1) normal acoustic presentation (2) processing through hearing aids (3) by direct activation by piezoelectric type devices. Further, pilot project results indicated the existence of differences between the hearing aid versus acoustic and PZT methods of presentation which were not accounted for by word discrimination tests alone. Therefore a new (listener) test paradigm including paired comparison quality ratings were instituted. Preliminary results indicate relative parity between the normal acoustic and direct ossicular chain activated (PZT) methods with both being superior to hearing aids. The differences appear to be dramatic in the quality rating category.

PROJECT DESCRIPTION

OBJECTIVES: Objective of this research was to quantify any differences which exist between inner ear response to speech initiated by normal acoustic means, by direct ossicular chain activation by piezoelectric transducers, and by passage through conventional hearing aid systems.

METHODS EMPLOYED:

1. Electrophysiological techniques used for recovery of speech from cochleas of live, anesthesized guinea pigs.
2. Identical tape recordings of phonetically balanced word lists used for each method of processing through guinea pig ear.
3. Processed lists presented to listener groups at 50 dB HL for word discrimination and quality ratings.

MAJOR FINDINGS: No statistical differences exist between cochlea responses to normal acoustic and direct ossicular chain coupled speech in terms of word discrimination. Both exceeded hearing aid processed speech.

Speech quality differences are presently being evaluated. Preliminary results indicate little "quality" difference between the normal acoustic and piezo-electrically initiated speech but vast differences between both and hearing aid processed speech.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings clearly demonstrate that direct ossicular chain coupled means of transducing speech onto the auditory system offer vast improvements in fidelity and freedom from distortion over that achievable by conventional hearing aids. Provided the implantation problems can be solved, which appears reasonable at this point, the method offers hope that improvements can be achieved in hearing prophalaxis for some individuals.

In experimental applications with laboratory animals, the device offers the opportunity to impart precisely controlled complex signals into the ossicular chain by direct electrical/mechanical transduction thus bypassing the difficult-to-control electro/acoustical step.

PROPOSED COURSE: Existing data will be analyzed and published. One additional experiment is planned. It's purpose is to test the intelligibility of speech initiated by direct driving of the bony shell of the cochlea itself.

PUBLICATIONS

Cook, R.O., Thomas, W.G., Konishi, T., Hamm, C.W. and Yankwich, A.H.: Speech discrimination derived from guinea pig CM initiated by ossicular chain coupled piezoelectric drivers, by hearing airs, and by conventional acoustic presentation. J. Acoust. Soc. Amer. 58(1): S104, 1975A.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50011-04 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Theoretical Basis of Dose-Response Relationships

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Phillip J. Walsh	Radiation Physicist	EBpB	NIEHS
Other:	Philip E. Hamrick	Senior Staff Fellow	EBpB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.8	1.1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to continue development of theoretical models of dose-response relationships for ionizing radiation at the cellular level and to begin the development of generalized conceptual schemes encompassing chemical, biological and physical agents. The current approach is to: (1) accumulate existing data for chemical, biological and physical agents on cell survival, mutagenesis and carcinogenesis that is suitable for dose-response analysis; (2) selection of models that can be applied to the data base in (1); (3) propose conceptual schemes within which further data can be accumulated to answer critical questions raised in (1) and (2).

PROJECT DESCRIPTION

OBJECTIVES: To examine whether generalized conceptual schemes can be proposed within which experimental and theoretical work can be directed to answer critical questions in dose-response analysis.

METHODS EMPLOYED: This project has included the development of a generalized theoretical model for the effects of ionizing radiation at the cellular level. A three-state model at the cellular level (normal cells, killed cells, altered cells) appears to encompass existing data and existing models on the effects of ionizing radiation. Such a model can be used to focus experimental work which, in turn, will lead to further model development. Questions to be addressed are: (1) whether, and at what level, models can incorporate effects of chemical, biological and physical agents; (2) whether paradigms for extrapolation can be proposed; (3) whether correspondence rules can be mechanistic (relate microevents to macroevents) or only phenomenological (macroevents only).

MAJOR FINDINGS: There appears to be no conceptual framework or theoretical model that can be used to provide guidance for investigation of environmental hazards in general. However, there exists extensive experimental data which has not been analyzed in terms of constructing a coherent theoretical model. Dosimetric concepts for chemical and biological agents are different from those for physical factors; particularly ionizing radiation, this is due to different interaction mechanisms that may require different conceptual models. Efforts to model dose-response relationships for ionizing radiation at the cellular level have been reasonably successful in spite of different approaches and underlying mechanistic uncertainty. It appears that the field of existing models (about 12-15) can be reduced to two or three with major similarities. Therefore a generalized theoretical approach with simplifying and synthesizing concepts appear warrented. It also appears reasonable to ask whether chemical and biological agents can also be incorporated into a general scheme.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Establishment of human exposure guidance is based upon extrapolation from existing data using theoretical models. It is expected that this will always be true. Thus confidence in extrapolation will increase in direct proportion to confidence in the basis of theoretical models.

PROPOSED COURSE: The proposed course is: (1) to accumulate existing data for chemical, biological and physical agents on cell survival, mutagenesis and carcinogenesis that is suitable for dose-response analysis; (3) provide a critical analysis as to the maturity of concepts of dose-response.

With results as a base, it should be possible to focus additional efforts. The work could be carried out by contract.

PUBLICATIONS

Walsh, P.J.: On the application of the uncertainty principle to stopping power theories. Health Physics 19:574, 1970.

Walsh, P.J.: Stopping power and range of alpha particles. Health Physics 19: 312-316, 1970.

Walsh, P.J.: Possible effects of dose rate and turnover time of cells on dose response relationships: Implications for radiation protecting guidance. International Journal of Environmental Studies 2:189-193, 1971.

Walsh, P.J.: Energy loss of alpha particles in tissue equivalent plastic. Health Physics 23:701-704, 1972.

Walsh, P.J.: Possible effects of division and repair of altered cells on dose response relationships in the context of carcinogenesis. International Journal of Environmental Studies 5:285-288, 1974.

Walsh, P.J.: On the relationship between cell survival and cell alteration after exposure on ionizing radiation. International Journal of Environmental Studies 8:1-11, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50013-06 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Variable Frequency Microwave Exposure System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Mechanical Engineer	EBpB	NIEHS
Others:	Phillip J. Walsh	Radiation Physicist	EBpB	NIEHS
	R. Mathews	Graduate Student		UNC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study was to develop a microwave exposure system which would provide a plane-wave type field and a variable frequency capability of 1-10 GHz. The system has been assembled and calibrated. The system generates a 90 percent uniform field over a 10 centimeter diameter circle with a power density capability range of 0-20 mW/cm^2. Small biological specimen can be exposed to variable frequencies in either a continuous wave or modulated wave mode.

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is to design, build and calibrate a variable frequency exposure system for biological material.

METHODS EMPLOYED: Specifications were written for the desired system. A field uniformity at the specimen of 90 percent over a 4-inch diameter circle, a continuous frequency variation in the range of 1 to 10 GHz and a power level range at the specimen of 0.010 to 10 mW/cm^2 were specified.

MAJOR FINDINGS: Many of the biological effects of microwave radiation are frequency dependent. In terms of whole body irradiation, a frequency of 150-1,200 MHz affects the internal organs, whereas, a frequency of 10,000 MHz and above affects primarily the skin. Components of cells such as RNA, DNA and protein were found to have maximum absorption at different frequencies. Therefore, it is necessary to have the capability of varying frequency when investigating microwave effects on biological systems. The frequency range of 1 to 10 GHz was chosen because it is within this range of frequencies to which the general population is exposed. The system has been assembled and calibrated. The system generates a 90 percent uniform field over a 10 centimeter diameter circle with a power density capability range of 0-20 mW/cm^2.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many biological effects of microwave radiation appear to be frequency specific. The variable frequency exposure system will provide the researchers of the Institute the capability of studying the effects of different frequencies and modulated signals on biological systems.

PROPOSED COURSE: The system is now being used to irradiate biological specimens. The project, therefore, has been completed.

PUBLICATIONS

McRee, D.I., Walsh, P.J., and Mathews, R.: Variable frequency exposure system for small biological specimens. Rev. Sci. Instruments 46:253-256, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50014-07 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Microwave Exposure Systems and Microwave Dosimetry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Mechanical Engineer	EBpB	NIEHS
Other:	Philip J. Walsh	Radiation Physicist	EBpB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

It is the objective of this project to develop exposure systems for bioeffects research and to develop and test techniques for measuring energy absorption. During the past year components have been obtained to develop waveguide exposure systems which will be used to expose cell systems and isolated neurons. The fabrication and calibration of the system will be accomplished during the next fiscal year.

The pressure transducer-capillary temperature detector was tested. The detector did not have the necessary stability at the desired sensitivity range. The response time of the instrument was slower than desired. As a result of these studies, this detector proved unsatisfactory for microwave energy absorption measurements at the levels associated with microwave hazards.

A liquid crystal-optic fiber (LCOF) temperature measurement probe was obtained from the Office of Naval Research. This probe does not interact with the microwave field and provides a technique for measuring energy absorption. We used the probe to test the thermistor which we have been using for energy absorption measurements. Excellent agreement between the two detectors was obtained and verifies our techniques as being accurate.

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to develop microwave exposure systems for biological material which have well-defined, field characteristics; and to develop dosimetric techniques for energy absorption.

METHODS EMPLOYED: A thorough study of the research requirements on the biological effects of microwaves and techniques for exposing biological specimens have been completed. Based on these studies, exposure systems will be designed and developed which provide known electromagnetic field characteristics. At present, it is difficult to accurately measure low level microwave fields and to determine the mechanisms by which the fields interact with matter. It is necessary, therefore, to conduct dosimetric studies so as to develop analytical interaction models. Microcalorimetry, thermistor and thermocouple detectors, chemical reactions, liquid crystals and photographic film are some of the techniques which will be investigated. Other techniques to determine energy absorption will be evaluated as they become available.

MAJOR FINDINGS: It has been determined that for the exposure of small biological specimens such as cell systems and isolated neurons, waveguide exposure systems offer excellent characteristics. During the past year components have been identified and obtained so that these waveguide systems can be developed in our laboratory. The pressure transducer-capillary temperature detector was tested. The detector did not have the necessary stability at the desired sensitivity range. The response time of the instrument was slower than desired. As a result of these studies, this detector proved unsatisfactory for microwave energy absorption measurements at the levels associated with microwave hazards.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The development of exposure systems with well-defined field characteristics will enable the Institute to perform accurate quantitative studies on the effects of microwave radiation on biological systems at frequencies ranging from 1-10 GHz. Techniques to determine the amount of energy absorbed will provide the capability of evaluating the data in terms of thermal or specific microwave effects.

PROPOSED COURSE: Waveguide exposure systems will be fabricated, calibrated, and operated during the next fiscal year. The development of a system to measure the electrical properties, dielectric constant and conductivity, of biological material will also be completed. A small thermistor probe, 1 mm in diameter, with projected accuracy of ±0.01°C and excellent response time has been developed by the National Bureau of Standards. This probe is said to have no interaction problems with microwave radiation. We plan to obtain an NBS thermistor probe and evaluate it in our laboratory.

PUBLICATIONS

McRee, D.I.: Determination of the absorption of microwave radiation by a biological specimen in a 2450 MHz microwave field. Health Physics 26:385-390, 1974.

McRee, D.I.: Determination of energy absorption of microwave radiation using the cooling curve technique. J. Microwave Power, 9(3): 263-270, 1974.

Huddleston, G.K. and McRee, D.I.: A pyroelectric probe for measurement of microwave power density under far-field conditions. Annals NY Acad. Sci., 247:510-526, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50015-03 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Microwaves on Neural Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Mechanical Engineer	EBpB NIEHS
Other:	Howard Wachtel	Associate Professor	Duke U.

COOPERATING UNITS (if any)

Duke University, Durham, North Carolina

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to determine the effect of microwave radiation on neurological response. Isolated neurons such as the abdominal ganglion of the Aplysia, the sciatic nerves of frogs and the saphenous nerves of cats will be exposed to CW and modulated microwave radiation in the power density range of 1-10 mW/cm^2. The effects of the microwave radiation on the strength-duration of a stimulus to produce an action potential, amplitude of the response, and conductive velocity will be investigated.

PROJECT DESCRIPTION

OBJECTIVE: The objective of this project is to determine the effects of microwave radiation on the functioning of the central nervous system when exposed to CW and modulated microwave radiation in the frequency range of 1-10 GHz.

METHODS EMPLOYED: Isolated neurons such as the abdominal ganglion of the Aplysia, the sciatic nerve of the frog, and the saphenous nerve of cats will be exposed to CW and modulated microwave radiation in the power density range of 1-10 mW/cm^2. The effects of the radiation on cell membrane potentials, action potentials and conduction velocity will be investigated.

More complex systems such as the exposed spinal cord of decerebrated cats will be exposed. These test systems will be used to investigate the effects of radiation on synaptic neurotransmitters.

MAJOR FINDINGS: A review of the literature indicates that the brain and central nervous system are sensitive to microwave radiation. In most studies, no explanation of the mechanism by which microwaves interact with nerve cells is specified. It has been determined that the best technique for exposing isolated neurons to microwaves, without interaction of the microwaves with the electrodes, is in a waveguide system. Components have been obtained to fabricate an exposure system for this research project.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential health effects of microwave radiation in the environment is of interest to NIEHS. The neurological and behavioral effects reported in the literature illustrate the need for significant effort in this area. At present, the accepted safe level of exposure in the U.S. is 1000 times greater than the standard in the USSR. The Soviet standard is based on neurological and behavioral response to microwave radiation. This research on the effects of microwave on CNS is directed toward the mission of the Institute to determine the health effects of physical factors in the environment.

PROPOSED COURSE: Neural specimens will be placed in a CW, pulsed, or modulated microwave field. The nerves will be stimulated and the effect of the microwaves on action potentials, conduction velocity and signal transmission will be measured. The strength-duration of the stimulation necessary to produce an action potential with and without microwave exposure will be an important endpoint. If effects are observed, the mechanism of interaction of the microwaves with the neural specimens will be investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50016-05 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of 2450 MHz Microwave Radiation on Biological Materials at Cellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Philip E. Hamrick	Senior Staff Fellow	EBpB NIEHS
Other:	Sherman S. Fox	Surgeon	EBpB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular and macromolecular level, to observe any effects of this interaction and to relate the amount of microwave energy absorbed to the effects. Biological systems employed in the study are: bacteria and bacteriophage, solutions of DNA, 2-4 cell stage embryos from mice, rabbit erythrocytes and rat lymphocytes. In these experiments, the temperature, humidity, and other various growth conditions have been carefully monitored in order to eliminate any effects not intrinsically caused by the microwave radiation. Results have shown very few differences between exposed and control samples that could not be explained by the thermal heating of the microwave radiation.

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to determine how 2450 MHz microwave radiation interacts with biological material at the cellular and macromolecular level, to observe any effects of this interaction and to relate the amount of microwave energy absorbed to the effects.

METHODS EMPLOYED: Various cellular and macromolecular systems are exposed to microwave radiation at carefully controlled temperatures and exposure levels. Much of the work has been at 37°C. Biological systems employed in the study are: bacteria and bacteriophage, solutions of DNA, 2-4 cell stage embryos from mice, rabbit erythrocytes and rat lymphocytes.

MAJOR FINDINGS: Studies by others have been made which suggest that "non-thermal" microwave fields (fields which do not significantly increase the temperature of the specimen) at various frequencies interact with intracellular components to alter metabolic and/or genetic processes. Interpretation of these effects is difficult because mechanisms are not specified and in many cases the exposure parameters are not known quantitatively. Many of the effects appear to be frequency dependent. For example, the growth rate of Escherichia coli has been shown by others to be enhanced or retarded depending on the frequency in the range of 65-75 GHz. In the past, when Escherichia coli and Pseudomonas aeruginosa were exposed in our lab to 2450 MHz microwaves at 60 mW/cm^2 and held at 37°C, no effects on growth rate were observed. Under similar conditions no effect was observed on the lysis time of bacteriophage T-2, or on the denaturation curves of DNA and no teratagenic effects were observed when mouse embryos were exposed. These studies have indicated that if there are "non-thermal" effects at 2450 MHz, then these effects are relatively small in the systems we have investigated. Polish and Russian scientists report effects on membrane transport in rabbit erythrocytes and stimulation of isolated lymphocytes to undergo transformation to a blastocyst-like cell. The study using rabbit erythrocytes has been repeated as nearly like the Polish work as possible. We were able to very closely duplicate the experiments since we had the opportunity to discuss the project with Polish scientists familiar with the work. Some of the parameters tested (such as K^+ efflux) were found to be highly temperature sensitive but no effects on membrane permeability were detected that were not due to temperature changes. During the past year, our work with rat lymphocytes in tissue culture has shown that there is no significant difference in stimulation when cells are exposed for 40 hours to 20 mW/cm^2 microwave radiation and in cells not exposed but held under similar conditions of temperature and humidity. These studies are being pursued at other exposure intensities and conditions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Before an accurate evaluation of the biological effects of 2450 MHz microwaves can be made, it is necessary to carefully control the temperature of the specimen and be able to reproduce the exposure conditions. We have the capability to do this at NIEHS and can distinguish observed effects of microwaves from purely thermal effects.

PROPOSED COURSE: We plan to continue our study with lymphocytes since stimulation would suggest immunological effects with possible profound

effects on animal systems. We will also study other systems in which sensitivity to microwaves has been reported or which are desirable as test systems for microwaves.

PUBLICATIONS

Hamrick, P.E., Thermal denaturation of DNA exposed to 2450 MHz microwave radiation, Radiation Research 56:400-404, 1973.

Hamrick, P.E. and Butler, B.T., Exposure of bacteria to 2450 MHz microwave radiation, J. Microwave Power 8:227-233, 1973.

Hamrick, P.E. and Zinkl, J.G., Exposure of rabbit erythrocytes to microwave radiation, Radiation Research 62:164-168, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 50017-03 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of 2450 MHz Microwaves on the Embryonic Development of Japanese Quail

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Mechanical Engineer	EBpB	NIEHS
	Philip E. Hamrick	Senior Staff Fellow	EBpB	NIEHS
Others:	J.P. Thaxton	Consultant	NCSU	
	C.R. Parkhurst	Consultant	NCSU	

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch, NIEHS
Poultry Science Department, North Carolina State University

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.6	0.7

SUMMARY OF WORK (200 words or less - underline keywords)

The exposure of fertilized Japanese quail eggs to 2.45 GHz CW microwave radiation for 4 hours per day for the first 5 days of development, and for 24 hours the second day of development to 30 mW/cm^2 power density have been completed. The newly-hatched quail were examined for changes in percent hatch, gross deformities, and hematological changes. No changes in hatchability or number of deformities were observed. A significantly lower level of hemoglobin was measured in the exposed birds. Fertilized quail eggs were also exposed for the first 12 days of development to 2450 MHz CW microwave radiation at a power density of 5 mW/cm^2. Again the effects on hatchability, number of deformities, and hematological parameters were measured in the 2 day old birds. In this series of tests, a small statistically significant increase in hemoglobin and large decrease in monocytes were measured. The above experiments were repeated but now the quail were kept to maturity and mated. It was found that the exposed male when mated with either the control or exposed female produce a 20 percent decrease in fertility. The exposed females when mated with control males produced no decrease in fertility. These birds were sacrificed at 22 weeks and changes in the size of the liver, bursa, and spleen were observed in the exposed birds.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are to determine the effects of 2450 MHz CW microwave radiation on the embryological development of Japanese quail and the subsequent growth, reproduction, and immunological response of the mature quail which had been exposed during the developmental period.

METHODS EMPLOYED: Fertilized Japanese quail eggs were exposed to 2450 MHz CW microwave for various periods of embryonic development at power densities of 30 mW/cm^2 and 5 mW/cm^2. The effects of exposure on hatchability, gross deformities and hematological parameters were measured in the 2 day old quail. Fertilized eggs were also exposed to 5 mW/cm^2 for the first 12 days of development. The quail were kept to maturity and growth rates, egg production, fertility and immunological response were measured.

MAJOR FINDINGS: The exposure of fertilized Japanese quail eggs to 2.45 GHz CW microwave radiation for 4 hours per day for the first 5 days of development, and for 24 hours the second day of development to 30 mW/cm^2 power density have been completed. The newly hatched quail were examined for changes in percent hatch, gross deformities, and hematological changes. No changes in hatchability or number of deformities were observed. A significantly lower level of hemoglobin was measured in the exposed birds.

Exposure of the fertilized eggs to a power density of 5 mW/cm^2 for the first 12 days of development has produced no gross deformities or changes in hatchability if the temperatures of the exposed eggs are maintained at the optimum incubation temperature during exposure. Hematological studies on the two day old chicks have shown a statistically significant increase in hemoglobin and decrease in monocyte percentage. Organ weights were also measured in these two day old chicks, and no significant differences between exposed and control quail were found. These experiments were repeated, but after hatching the quail were allowed to grow to maturity. These mature quail were mated and eggs were incubated in a standard hatching incubator. It was found that the exposed males when mated with either the control or exposed females produced a 20 percent decrease in fertility. The exposed females when mated with the control males resulted in the same fertility rate as the mating of the control females with the control males. At the end of 22 weeks, the treated and control quail were sacrificed, examined and organs weighed. The testes, spleen, bursa, adrenals, liver and heart weights were compared. No significant difference in the weights of the testes, adrenals, and heart were observed between the irradiated and the non-irradiated quail. No differences were found in the weights of the spleen and bursa of the irradiated female quail when compared with the non-irradiated female quail. However, there was a large increase in the spleen and bursa of the treated males when compared to the untreated males. The weights of the livers of the exposed female quail were significantly smaller than the livers of the non-exposed. In approximately 75 percent of the treated females, one lobe of the liver was noticeably smaller than the other lobe.

In summary, these results show that although the neonate quail which were exposed to microwaves during development showed only small changes in hemoglobin and monocyte percentage, the reduction in fertility and changes in organ weights in the mature quail are significant effects. The reductions in fertility indicate

either a reduction in viable sperm or change in the mating behavior. In the bird, the bursa is the site of the production of B cells which move through the blood stream to the spleen to form clones. These clones in the spleen produce approximately 90 percent of the antibodies in the quail. Since the size of the bursa and spleen were both larger in the treated males, it is probable that the immunological response of these quail would be enhanced when compared to the untreated males. The smaller malformed livers of the treated females would have a detrimental effect on the general health of these quail.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evaluation of whether exposure to microwaves during embryonic development has an effect on the developmental process and function of the organism after maturity must be answered before microwave radiation exposure can be considered safe. This project will assist the Institute in its mission of determining the health effects of physical factors in the environment.

PROPOSED COURSE: Experiments to determine the cause of the decrease in fertility due to the effects on the male quail will be performed by studying mating behavior and the sperm produced by these quail. Pathological studies of the organ tissues will be performed to evaluate the effects on organs; particularly the liver, spleen, and bursa. Immunological studies will be accomplished by determining the ability of the 22 week old quail to produce antibodies in response to a challenge by sheep red blood cells.

PUBLICATIONS

McRee, D.I., Hamrick, P.E., Zinkl, J.G., Thaxton, J.P., and Parkhurst, C.R.: Some effects of exposure of the coturnix quail embryo to 2.45 GHz microwave radiation. Ann. NY Acad. Sci., 247:377-390, 1975.

Hamrick, P.E., McRee, D.I., Zinkl, J.G., Thaxton, J.P., and Parkhurst, C.R.: Hematology of neonatal Japanese quail. Lab. Animal Sci. 24:495-499, 1975.

Hamrick, P.E., Zinkl, J.G., McRee, D.I., Thaxton, J.P., and Parkhurst, C.R.: Leucopenia in neonatal Japanese quail. Poultry Science 54:312-314, 1975.

Hamrick, P.E. and McRee, D.I.: Exposure of the Japanese quail embryo to 2.45 GHz microwave radiation during the second day of development. J. Microwave Power 10(2):211-221, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50018-01 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Investigation of Microwave Radiation Effects on Neurological Functioning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald I. McRee	Mechanical Engineer	EBpB NIEHS
	George Somjen	Consultant	Duke U.

COOPERATING UNITS (if any)

Duke University, Durham, North Carolina

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The spinal cord of cats was directly exposed to 2450 CW microwave radiation in order to study the effect on reflex response and synaptic function. A small but statistically significant increase in the reflex response was detected in the first series of experiments, which indicates enhancement of the synaptic transmission. However, this effect was not observed in a second series of experiments in which the incident power density was increased from 10 mW/cm^2 to 20 mW/cm^2 and a more rigorous experimental design was employed. The slight changes that were observed in the second series could be attributed to small temperature variations during the experiment.

PROJECT DESCRIPTION

OBJECTIVES: The objective of the research is to investigate the non-thermal effects of microwave radiation on neurological functioning. The spinal cord of cats which were functionally decapitated were used as test specimens.

MAJOR FINDINGS: The spinal cords of cats were exposed to 2.45 GHz CW microwave radiation of 10 mW/cm^2 and 20 mW/cm^2 power density, and the effect on the reflex response and synaptic transmission were measured. It was found that a small but statistically significant increase in the amplitude of the response was measured during microwave exposure. Since temperature of the cord could only be controlled to within ±0.2°C, the increase in response could possibly be due to the specific heating of the cord during microwave exposure. Whether the effect is thermal or due to the direct interaction of the electromagnetic fields, the result is significant because of the ability of microwaves to penetrate tissue and interact with neurons below the skin. Since low temperature changes in neurons are known to produce an effect on the action potential, this work illustrates the ability of low level microwaves to produce such an effect whether it be thermal or non-thermal.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The effects of microwave radiation on the central nervous systems and behavior have been reported frequently in the literature. It is important to determine if levels of radiation in the environment will produce such effects. The research will assist in the establishment of levels of exposure which are safe for the general population.

PROPOSED COURSE: This project has been completed. Any future research using the spinal cord of the cat as a test specimen will be included in another project.

PUBLICATION

McRee, D.I., Wyatt, R.H., Haseman, J.K. and Somjen, G.: The transmission of reflexes in the spinal cord of cats during direct irradiation with microwaves. J. Microwave Power (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50019-01 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effect of Ototoxic Insult on Coding of Complex Signals in the Auditory System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Sanitary Engineer	EBpB	NIEHS
	Teruzo Konishi	Visiting Scientist	EBpB	NIEHS
Others:	Charles W. Hamm	Physical Science Tech.	EBpB	NIEHS
	Stanley Hutcheson	Consultant	CA	UNC

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.35	.65	.70

SUMMARY OF WORK (200 words or less - underline keywords)

Results of behavioral and audiological tests with humans imply that ability to understand speech and other complex signals (particularly in a noisy listening situation) suffers loss from noise insult in excess of what could be inferred from pure tone thresholds measured in the quiet. These findings suggest that a complex interaction between the various levels of the auditory nervous system and between the auditory nervous system and functionally different receptors of the auditory end organ play a vital role in "sharpening" the sensory process.

Until recently it has not been possible to study the effect of noise insult on rapidly changing speech or speech-like signals because such study necessitated the use of extremely fast-acting signal analysis (frequency domain) equipment coupled with the memory capacity of a minicomputer.

The PDP 1140 Minicomputer in our lab is being interfaced with signal analysis equipment for processing electrophysiological data by Fast Fourier Transformation, Auto and Cross Correlation analysis. The responses from both multi cochlear microphonic(CM) and responses from individual nerve fibers of noise exposed and non-noise exposed animals will be obtained.

PROJECT DESCRIPTION

OBJECTIVES: Objective of this work is to demonstrate whether physiological responses to complex speech-like signals are a more sensitive measure of noise insult than conventional CM pure tone loss. This project is viewed as having the potential for being expanded, as our ability to handle and analyze large amounts of data in real time improves. Investigation of critical ratio and other concepts are forseen.

METHODS EMPLOYED: Minicomputer, signal analysis equipment, pseudo random noise, electrophysiological techniques, initial noise exposure would be band limited white noise

MAJOR FINDINGS: Program is in initial stages, efforts so far have been concerned with programming and debugging the computer.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In recent years, it has become clear that the ability of the cochlear transducer to respond to pure tones is not an adequate indicator of its ability to respond to the natural complex stimuli present in the real world. Studies of the former are legion, studies in the latter area, particularly after ototoxic insult, are nearly nonexistant in spite of the fact that complex stimuli perceived in a noisy environment constitute the natural situation. We hope to obtain data which will begin to fill this void.

PROPOSED COURSE: Project will be approached as outlined above.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50020-01 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of a Fiber Optic/Photodetector System for Basilar Membrane Measures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Reginald O. Cook	Sanitary Engineer	EBpB	NIEHS
Others:	Charles Hamm	Physical Science Technician	EBpB	NIEHS
	Teruzo Konishi	Visiting Scientist	EBpB	NIEHS
	Earl Schubert	Professor	HSS	SU

COOPERATING UNITS (if any)

Hearing and Speech Sciences, Stanford University, Palo Alto, California

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

SUMMARY OF WORK (200 words or less - underline keywords)

When the ossicular chain displaces in response to a sound, a hydrodynamic pressure wave is initiated in the cochlea which is thought to cause the basilar membrane to respond in a manner dependent upon the sound's amplitude and frequency content with the low frequency peaks occurring spatially removed from highs. Uncertainties regarding the high and low frequency slopes of the maximas as well as whether a plateau effect occurs at high intensities presently block completion of hearing theories at the intracochlea mechanical level. That the technical difficulties associated with the obtaining of these measures are formidable is a commonplace in the auditory field. However, on the basis of a thorough theoretical analysis of the relevant parameters, it is believed that the electro-optic technology has advanced to the point where a reasonable chance of success exists. While other methods; i.e, laser interferometer, optical heterodyneing spectroscopy, may be an order of magnitude more sensitive on a theoretical basis, none approach the fiber optic lever in simplicity of use. In addition, it is the only method which allows instantaneous waveform recovery. A theoretical waveform recovery sensitivity in the 1-10 angstrom range with a seven fiber probe can be deduced from the theoretical analysis performed. Achievement of such levels in an operational probe will require interactive improvement of prototypes.

PROJECT DESCRIPTION

OBJECTIVES: To develop a DC to 20 KHz audio frequency displacement sensor capable of resolving 1-10 angstroms, and in a configuration from which displacements along parts of the basilar membrane can be measured.

METHODS EMPLOYED: Physics, electro optics, optics (fibers), electronic engineering.

MAJOR FINDINGS: A theoretical analysis performed in our laboratory revealed that 1-10 angstrom resolution at a S/N ratio of one was possible under an optimized configuration. Prototypes are being built to determine whether this level is achievable in practice.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Inability to measure the actual displacement of the basilar membrane has been a stumbling block to understanding the intracochlear signal transduction mechanism. It is hoped that with this measuring scheme some of the missing data links can be filled.

PROPOSED COURSE: In development of projects which push the state of the art, the best approach is usually iterative prototype development, where attempts are made to solve only one set of problems per stage. It is proposed to follow this paradigm.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 50021-01 EBpB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Ultrastructure of Hair Cell-Nerve Junction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruzo Konishi	Visiting Scientist	EBpB	NIEHS
	Robert Gulley		LNO	NINCDS
Others:	Reginald Cook	Sanitary Engineer	EBpB	NIEHS
	T.S. Reese	Head, Functional Neuroanatomy	LNNS	NINCDS
	Alexandra Yankwich	Health Services Officer	EBpB	NIEHS
	G. Neises		LNO	NINCDS

COOPERATING UNITS (if any)
Laboratory of Neuro-otolaryngology and Laboratory Neuropathology and Neuroanatomical Sciences, National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH
Environmental Biophysics Branch

SECTION
None

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study changes in the ultrastructure of the hair cells of the organ of Corti. The present project is designed to study the ultrastructural and electrophysiological changes of hair cells after alteration of the ionic concentration of endolymph in the guinea pig cochlea.

PHS-6040

PROJECT DESCRIPTION

OBJECTIVES: Dr. Robert Gulley found that in the outer hair cells in the chinchilla, there is no accumulation of synaptic vesicles in the presynaptic membrane, although EM structure demonstrates the chemical synapse. The purpose of this project is to examine the ultrastructural and electrophysiological changes in hair cells by alteration of the ionic concentration of the endolymph in the chinchilla and guinea pig.

METHODS EMPLOYED: Stimulus-related responses were recorded with differential electrodes in the basal turn of anesthetized chinchillas. The endocochlear potential was also recorded from the scala media. Using a microperfusion technique, the endolymphatic space was perfused with artificial perilymph or isotonic KCl solution over a period of 20 minutes. The amount of perfusate ranged from 3 to 5 µl. Immediately after perfusion the cochlea was prepared for EM study.

MAJOR FINDINGS: Control animals were perfused with isotonic KCl solution. The electrical responses were not markedly affected by the slow perfusion of the scala media with isotonic KCl. The endolymphatic space was intact and there was no hair cell loss and no breakdown of intercellular junctions. The synaptic region was normal with no accumulation of vesicular structures near the synaptic region.

Animals perfused with artificial perilymph showed a progressive decline of cochlear microphonics and action potential during perfusion. The endocochlear potential decreased in magnitude as perfusion progressed. The number of vesicular structures in the synaptic region of the outer hair cell were increased but there was no clear increase in the number of vesicles associated with the presynaptic membrane.

Animals perfused with artificial perilymph and kept under relatively quiet conditions showed similar EM changes to those observed after perfusion with perilymph.

The changes observed are not consistent with any single hypothesis of synaptic function and only indicate that the synapses in the organ of Corti can be manipulated by experimental changes in endolymph compositon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The mechanism involved in synaptic transmission at the hair cell-nerve junction has been understood. The results obtained through this project will give us significant information concerning the dynamic characteristics of the hair cell-nerve junction. This information is important for an understanding of functional changes induced by noise exposure or by ototoxic agents.

PROPOSED COURSE: Additional experiments, such as stimulating the efferent fibers with and without changing the endolymph, perfusing the perilymph with increased Mg^{++} and lowered Ca^{++}, and endolymphatic perfusion with no K^{+} and Na^{+} will be done in the near future.

ENVIRONMENTAL MUTAGENESIS BRANCH

ENVIRONMENTAL MUTAGENESIS BRANCH
Summary Statement

The primary objective of the Institute's program in mutagenesis is focused on evaluation and definition of risks to both human somatic and germinal tissue resulting from exposure to genetically active environmental chemicals. The Environmental Mutagenesis Branch was organized late in FY72. Because of the worldwide shortage of resources for the rapid development of research in various problem areas associated with environmental mutagenesis, staff members have used a variety of approaches to accomplish its objective. These include:

1. Development of an intramural research program divided into five main areas. Work on particular projects has been carried out by different mechanisms:

   a. in-house research by staff scientists

   b. support of work at other institutions in the collaborative research program with staff scientists as project officers

   c. developing an announcement in collaboration with the extramural program office to solicit grant proposals in specific emphasis areas of environmental mutagenesis

2. Development of a coordinated national program in environmental mutagenesis. A variety of mechanisms have been initiated including:

   a. formation of a DHEW Subcommittee on Environmental Mutagenesis (SEM)

   b. developing workshops and conferences in special emphasis areas

   c. developing collaborative programs with other institutes within NIH and with other agencies

   d. developing programs within the Environmental Mutagen Society in which staff members serve as officers, councillors or members

   e. developing collaborative studies on genetically active environmental chemicals to rapidly develop a data base to assist in evaluating the risk of exposure to man

3. Development of better communication and coordination at the International level. Staff members are associated with several international programs including:

   a. US-Japan Cooperative Medical Science Program

   b. US-USSR Environmental Protection Agreement

   c. US-Germany Life Sciences Program

d. development of international workshops and conferences

e. collaboration with IARC (Lyon) on the mutagenicity of chemical carcinogens

4. Development of better mechanisms for information exchange. These include:

   a. support of the Environmental Mutagen Information Center (EMIC)

   b. serving as editor, associte editor or members of the editorial board on journals which publish research in the area of environmental mutagenesis

5. Development of better mechanisms for training young scientists for research careers in environmental mutagenesis at:

   a. the predoctoral level

   b. the postdoctoral level

INTRAMURAL RESEARCH PROGRAM

The intramural staff has been organized into five sections. The major research accomplishments in each section are as follows:

The Microbial and Plant Genetics Section has made considerable progress in the development and improvement of simple and comprehensive tests for gene mutations and other types of genetic damage in microorganisms. Dr. Tong-man Ong has developed a spot test system for detecting reverse mutations at the ad-3 locus of Neurospora crassa. Various tester strains have been selected that exhibit sensitivity to various types of chemical mutagens. This rapid assay will complement the ad-3 forward mutation system earlier developed by Dr. de Serres which has been used by Drs. Ong and de Serres to test the mutagenicity of several antischistosomal agents, chemical carcinogens and a wide variety of environmental chemicals. These studies are designed primarily as structure-function analyses to determine the correlation between types of genetic damage induced by compounds of similar structure.

Dr. de Serres is also continuing studies on repair-deficient strains of Neurospora. There is similarity in the spontaneous mutation rate at the ad-3 locus with both the wild-type and the repair-deficient strains; however, with x-irradiation, the repair-deficient strain required a 5- to 8-fold reduction in dose to give the same induced frequency as the wild-type Neurospora. In additional studies with repair-deficient strains, Drs. Inoue, Ong and de Serres have shown a variety of sensitivities of various strains to the chemical carcinogen ICR-170. The relative mutagenic responses of the UV-sensitive strains and the wild-type to ICR-170 are different from the relative response observed with UV, x-rays and MNNG.

Studies with various microbial systems have further strengthened the correlation between carcinogenic and mutagenic activity. The ad-3 data indicate that the carcinogens tested produce mutations primarily by base-pair substitutions. The utility of microbial mutagenesis assays as a prescreen for chemical carcinogens appears to be very promising.

During the past year, the Somatic Cell Genetics Section has focused attention on the interaction of mutagens and carcinogens with DNA and synthetic polynucleotides. In one study on the binding of heavy metals it is indicated that different cations may exhibit selectivity in binding to DNA. Additionally, studies were conducted to measure the binding of chemical mutagens and carcinogens with the goal of a molecular understanding of the molecular basis of mutagens.

Drs. Amacher, Elliott and Lieberman are studying DNA repair synthesis in a variety of mammalian cell lines after exposure to acetylaminofluorene. While all cell lines remove substantial amounts of the bound mutagen, there are differences in rates of repair which are being further studied. In conjunction with this, Dr. Lieberman is collaborating with Dr. Gary Stein of the University of Florida to study nuclear protein synthesis in cultured human cells after exposure to chemical mutagens. Repair induced by UV and acetylaminofluorene progresses without new histone or acidic protein synthesis.

Dr. Huang is developing a gene mutation detection system in human diploid fibroblasts. Forward mutations will be detected at a sex-linked locus (HGPRT) and an autosomal locus (AGPRT). Drs. Huang, Elliott and Ishii will also examine mutagenesis and in vitro transformation in the same cell lines to determine the relationship of mutagenesis to the oncogenic process. Dr. Lieberman is collaborating with Dr. Darrell Stafford of the University of North Carolina in a study to isolate the ribosomal DNA cistron from human cells.

Studies in the Biochemical Genetics Section utilize a variety of approaches to develop new assay systems to detect gene mutations in mammalian somatic and germ cells. Drs. Valcovic and Malling have developed a biochemical specific locus test based on electrophoretically detectable enzyme loci. The system can detect mutations that result in either a non-functional enzyme or a functional protein with altered electrophoretic mobility. Results to date from a ^{60}Co irradiation experiment indicate that the assay is at least as sensitive as the morphological specific locus system. The advantages of the new system are (1) the number of loci per animal tested can be easily increased, (2) since many of the enzymes are also in the human population, it will provide a base-line for monitoring of humans for increased mutation frequency. Drs. Mareschal and Valcovic are also investigating the heat-stability of mouse enzymes to determine the utility of this endpoint as an indicator for induced mutation. It is possible that this could be incorporated into the electrophoresis assay and, hence, further increase the number of genetic loci tested.

Considerable progress has been obtained by Drs. Mitra, Elkington and Malling in the development of a mutation detection system in mammalian sperm. Such a system is important not only for determining mutagenic activity of environmental chemicals, but also for monitoring human males in high hazard industrial

situations. Sperm cells which stain under selective conditions would be presumptive mutants. Test conditions have been defined for three enzymes and validation studies are underway.

In a correlative area Dr. Maier and Dr. Malling are developing a system using a mammalian testes microsomal activation system. This is similar to the liver microsome system with bacterial testers which is in widespread use but offers the advantage of using testicular tissue, the crucial tissue of concern for hereditary mutations.

Successful refinement of the liver microsomal activating system coupled with bacterial tester strains has been utilized to initiate a program using a computer programmed mass screening system. This project will attempt to provide standardized protocols and computerized control of tests and data evaluation.

During the past year studies in the Mammalian Genetics and Cytogenetics Section have focused on the comparative sensitivities of different mouse strains to mutagenic treatment. Drs. Sheridan and Soares are comparing the induction of dominant lethal mutations induced in a wide variety of inbred strains of mice after treatment with a given dose of triethylenemelamine. The objective of this work is to develop a battery of tester strains ranging from very sensitive to very resistant which will better mimic the range of variation that we can expect in the heterogeneous human population.

In order to study the apparent lack of response of mouse spermatogonial stem cells to mutagenic treatment, Drs. Sheridan and Soares have used combined treatments of radiation and chemical mutagens and are investigating whether or not pretreatment with irradiation will sensitize spermatogonia to induction of mutations by chemicals. In addition, Dr. Soares, together with Drs. Matthews, Eling and Anderson of the Pharmacology Branch, using radiolabeled mutagen, has studied the distribution of the chemical throughout various body tissues.

A study to detect induced genetic changes in both somatic and germ cells is being carried on by Drs. Soares and Sheridan. Both lymphocytes and sperm from chemically treated males are being examined for chromosomal aberrations. The aim of this approach is to use somatic cell mutagenesis as a predictor for germ cell effects. The success of this program could greatly reduce the cost and time of mammalian mutagenicity testing.

Drs. Burki and Sheridan have studied chromosome aberrations in early mouse embryos after mutagenic treatment of parents. While dominant lethal effects have been induced by several chemicals, the genetic causes have not been elucidated. This research seeks to determine the relative contribution of chromosome aberrations to this class of dominant lethal events.

Studies in the Population Genetics Section are in progress to compare the spontaneous mutation rates in natural and laboratory environments. In collaboration with scientists in the Environmental Biometry Branch and North Carolina State University, studies analyzing changes in gene frequency in response to exposure to various environmental chemicals are being carried out. These experiments involve monitoring gene frequencies of experimental Drosophila populations as they are exposed to various toxic environmental chemicals.

Drs. Gillespie and Langley, with Dr. Guess of the Environmental Biometry Branch, have been developing mathematical models to describe the effect of migration on gene frequencies. This program attempts to answer basic questions about the role of geographic distribution of populations and migration upon the establishment and maintenance of genetic variability.

Efforts to establish low dose risk estimates from animal carcinogenesis experiments are being carried out by Drs. Langley and Hoel (Environmental Biometry Branch). Most laboratory studies use high doses of chemical carcinogens; and since humans may more often be exposed to low doses, it is important to determine low dose effects. This approach uses various models of carcinogenesis to generate dose response curves for low dose extrapolation.

Collaborative Research Program - In the Collaborative Research Program two contracts were initiated in FY74 to detect gene mutations in somatic cells and germ cells in mammals. In the first contract at the University of Washington an attempt is being made to develop a simple system to measure point mutations in readily accessible human somatic cells. This assay is based on changes in the expected frequency of human red blood cells containing fetal hemoglobin rather than adult hemoglobin. Specific antisera are being developed to detect mutations which produce further A_γ or G_γ fetal hemoglobin. By conjugating the two antisera with different colored fluorescent dyes, mutant RBC's will fluoresce in only one color rather than two. In another contract at the Jackson Laboratory progress has been made toward the development of a new mouse strain carrying multiple chromosome inversions to make it possible to detect gene mutations (recessive lethal mutations) in the chromosome regions covered by the inversions. One of the 31 inversions thus far recovered has been used in tests to detect x-ray induced recessive lethal mutations to demonstrate the general utility of this approach.

A second contract with the Jackson Laboratory involves the incorporation of 13 additional mouse enzyme alleles into the C57BL/6J strain. This will directly complement the electrophoretic assay system developed at our Institute and will significantly enhance the sensitivity of the system to detect biochemical specific locus mutations.

In a new contract initiated in the past year with the Georgia Institute of Technology attempts are being made to develop a polygenic assay for the detection of point mutations in mice. By studying changes in the means and variances of characters determined by many genes, it should be possible to detect genetic changes which do not express major phenotypic effects.

In another new contract with Knox College studies are being conducted in Drosophila melanogaster to determine the effects of dietary deficiency on the sensitivity to mutagenic treatment. Preliminary results have indicated that some deficiencies increase the amount of genetic damage observed following treatment.

In another contract at the University of Wisconsin an assay is being designed to assay the induction of gene mutations at two different loci, HGPRT and AGPRT. During the past year, dose response curves have been obtained after treatment with two potent mutagens and carcinogens, MNNG and nitrosofluorene. The

genetic and biochemical characterization of over 100 independently induced and spontaneous mutant cell lines has shown that the base-analog resistant mutants obtained with this assay are stable genetic variants and not transient phenocopies of some sort.

We have continued to support a contract at Miles Laboratory to perform the genetic analysis of ad-3 mutants recovered from experiments on haploid strains of two-component heterokaryons. During the past two years we have also been using repair-deficient strains of Neurospora, as the genetic characterization performed in this manner has shown striking qualitative differences between wild-type and excision repair-deficient strains.

By interagency agreement, we are supporting base-line studies to determine the mutagenicity of airborne chemical pollutants and other gases used by industry. Various clones of the plant Tradescantia paludosa are being developed to select one particularly sensitive clone for assays of the genetic activity of weak mutagens. Field studies are being planned during FY76 in collaboration with NERC-EPA to compare the effects of exposure to the same airborne pollutants under laboratory and field conditions.

During the past year we have also continued to support EMIC (Environmental Mutagen Information Center) by interagency agreement at the Oak Ridge National Laboratory. This center has over 16,300 bibliographic entries in its data banks and contains as a unique world-wide resource for information in the area of environmental chemical mutagenesis.

NATIONAL PROGRAMS

To develop better coordination in environmental mutagenesis and to provide perspective for the intramural scientific staff in problem definition and resolution in the rapidly developing field, a variety of mechanisms have been developed.

SEM - (DHEW Subcommittee on Environmental Mutagenesis, chaired by Dr. F. J. de Serres, Chief, Environmental Mutagenesis Branch, NIEHS). Intramural staff members have continued to hold meetings with at least 20-30 representatives from DHEW and other government agencies to develop better communication within DHEW as well as between other agencies with programs in environmental mutagenesis. The Panel meets monthly for discussion, and minutes are recorded to provide the basis for furthering communication within the various member institutes and agencies. The minutes of all meetings are included in a yearly report to the parent DHEW Committee to Coordinate Toxicology and Related Programs (chaired by Dr. D. P. Rall, Director, NIEHS).

Workshops and Conferences - In collaboration with the Pharmacology Branch, a conference on "In Vitro Metabolic Activation" was held on February 9-11, 1976, at Burroughs Wellcome. The proceedings will be published by Elsevier.

Collaborative Programs with Other Institutes within DHEW and with Other Government Agencies

1. NCI - In collaboration with staff scientists at NCI, a collaborative research program has been persued to determine the correlation between carcinogenic and mutagenic activity of chemical carcinogens and non-carcinogenic structural analogs. This has also involved development of mechanisms to review and evaluate performance of the contractors as well as the planning of workshops to evaluate the accomplishments of the overall program. This aspect of the program was completed during FY76. In collaboration with staff members of the In Vitro Carcinogenesis Program, new plans have been made to validate microbial and mammalian tests for mutagenicity for use as prescreens in the carcinogenicity test program.

2. FDA - In collaboration with staff scientists in the Bureau of Foods, NIEHS staff scientists have participated in the successful development and review of collaborative research programs to perform mutagenicity tests on food additives.

3. EPA - In collaboration with staff scientists in the Office of Toxic Substances, NIEHS staff scientists have participated in the development of collaborative research programs to perform mutagenicity tests on pesticides.

4. NSF and Other Agencies - Staff scientists have participated in the review of proposals for contract work and in site visits to select suitable contractors for research in the area of mutagenicity testing.

5. NIDA - Staff scientists have participated in the review of proposals and in evaluating the performance of contractors in connection with mutagenicity tests on chemicals used in drug abuse treatment.

Collaborative Studies on Genetically Active Environmental Chemicals - During FY76 a collaborative study to evaluate the mutagenicity of commercially available hair dye components was continued. This study was initiated to confirm and extend the observations of Ames and others on Salmonella to other indicator organisms. A similar study has been started during FY76 on the flame retardant "tris." Collaborative studies of this type provide basic data with regard to both quantitative and qualitative effects of genetically active compounds over a wide range of organisms. Such data is essential for a meaningful risk analysis with regard to human exposure.

INTERNATIONAL PROGRAM

Staff members are associated with a wide variety of international programs with individual countries as well as with international agencies and programs.

US-Japan Cooperative Medical Sciences Program - In this program the U.S. Environmental Panel (Chairman, F. J. de Serres, Chief, Environmental Mutagenesis Branch, NIEHS) organized a conference on "In Vitro Mutagenicity and Carcinogenicity Tests" which was held in Seattle, Washington, on July 21-24, 1975. The topics discussed were as follows: qualitative correlation between

carcinogenicity and mutagenicity. General criteria to determine mutagenic potential in experimental organisms Current status of testing programs on the mutagenicity of chemical carcinogens. Correlation between carcinogenicity and unscheduled DNA synthesis and DNA repair. Current status of testing programs in the area of in vitro mutagenesis and carcinogenesis.

US-USSR Environmental Protection Agreement - In connection with this agreement the third workshop on Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis was held in Dushanbe, Tajikistan, on April 6-8, 1976. The program was focused on research directed towards developing better screening systems to detect mutagenic and carcinogenic activity as well as methods suitable for genetic monitoring of the human population.

US-Germany Life Sciences Program - In connection with the agreement with the Federal Republic of Germany, various NIEHS staff members have consulted with staff members at the Gesellschaft fur Strahlen-und Umwelt. A collaborative study is being developed to study electrophoretic variants resulting from gene mutations in the germ cells of mice.

Collaboration with IARC (Lyon) - During FY76 a new program was continued which involves participation between the National Institute of Environmental Health Sciences staff scientists, EMIC personnel and IARC scientists to translate mutagenicity data of known chemical carcinogens. Staff members are also participating in a series of workshops organized by the IARC staff to evaluate the correlation between carcinogenic and mutagenic activity and the possibility of using short-term tests for mutagenic activity to predict potential mutagenic and carcinogenic activity in man.

Collaboration with the Laboratory of Radiation Genetics and Environmental Mutagenesis (Leiden) - A collaborative program to study the mutagenicity of known chemical carcinogens has continued. It involves a comparison of the mutagenicity of chemical carcinogens in Drosophila and Neurospora. The studies with Drosophila have shown that the one lesion produced by all chemical carcinogens is recessive lethal mutations; the objective of the Neurospora studies is to confirm and extend these observations by the identification of carcinogen-induced lesions at the molecular level.

International Symposia, Workshops, Meetings - An International Symposium on New Developments in Mutagenicity Testing of Environmental Chemicals was held in Zinkovy Castle, Czechoslovakia, on October 13-17, 1975. The meeting was attended by Drs. F. J. de Serres, H. V. Malling and G. R. Mohn from NIEHS, as well as other scientists from the United States, Europe and the Soviet Union. The main topic areas were overview, correlation between mutagenicity and carcinogenicity, mutagenicity testing and toxicology, case studies using different testing systems and human population studies. These were followed by panel discussions on mutagenicity testing - submammalian systems, mutagenicity testing - mammalian systems and estimation of risk.

Following this Symposium Drs. de Serres, Malling and Mohn attended the Vth Annual Meeting of the European Environmental Mutagen Society in Florence, Italy, on October 19-22, 1975. Dr. de Serres gave the Opening Address entitled "Prospects for a revolution in the methods of toxicological evaluation." Both Drs. Malling and Mohn presented contributed papers.

An international conference organized by the Institute de la Vie on "Birth Defects: Detection and Prevention" in Trois Islets, Martinique, on January 26-28, 1976, was attended by Dr. F. J. de Serres, in which he presented a paper and led a panel discussion on Similarities and Differences between Carcinogens, Mutagens and Teratogens.

INFORMATION EXCHANGE

EMIC (Environmental Mutagen Information Center) - The Institute has continued to support the work of EMIC to collect, organize and disseminate information on environmental chemical mutagens. The Center not only processes requests for information from various government agencies, but is now becoming well known abroad. During the past year, EMIC's data file reached 16,300 bibliographic entries.

Journals - Staff members serve as associate editors, assistant editors or members of editorial boards of Mutation Research, Cancer Research, Environmental and Experimental Botany, The Journal of Toxicology and Environmental Health Perspectives. This makes it possible not only to promote publication of papers dealing with various research areas of environmental mutagenesis but also to promote development of new methods for publication.

TRAINING PROGRAMS

An International Course on Environmental Mutagenesis was held in Erice, Sicily, on October 24-31, 1975, in which both Drs. Malling and de Serres participated as lecturers. The course was attended by 47 students from Italy, Japan, Kenya, Sweden, Switzerland, United Kingdom, United States and Yugoslovia.

The great shortage of scientists to do research in the area of environmental mutagenesis both in the United States and abroad has provided incentive for staff scientists to develop a training program at both the predoctoral and postdoctoral levels. During the past year, we have provided facilities for research for three predoctoral students from the University of North Carolina at Chapel Hill. At the postdoctoral level, staff scientists include five staff fellows and eleven visiting fellows, one visiting associate and one visiting scientist. In the visiting program, postdoctoral fellows in training have come from Switzerland (2), Belgium (1), Federal Republic of Germany (2), Australia (2), Japan (3), England (1), India (1), Italy (2) and Taiwan (1).

In conclusion, during the past year we have been successful in recruiting key personnel for our intramural program, have continued cooperative programs with other Branches in the Institute, have coordinated program development of the Branch with complementary programs in other Federal agencies, and have established lines of communication with newly developing scientific organizations for environmental mutagenesis abroad. Along with the organization of workshops and conferences, these approaches will be used to stimulate research and development in this rapidly developing area of environmental health science.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60001-02 EMB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mutation Induction in Repair-Deficient Two-Component Heterokaryons of Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. J. de Serres	Branch Chief	EMB NIEHS
OTHER:	N. J. Bernheim	Microbiologist	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Mutagenesis Branch

SECTION

Microbial Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to determine the effect of various repair-deficient strains on the process of mutation-induction at specific loci in Neurospora crassa.

Using the ad-3 test system in a two-component heterokaryon, specific locus mutations can be recovered which result from both point mutations and chromosome deletions; this latter class is haplo-lethal and cannot be recovered in experiments on haploid strains. Various studies have shown that with variation in dose-rate with x-rays, photo-reactivation after UV, etc., that the lesions giving rise to these two classes of mutations are qualitatively different. The objective of this work is to determine whether upr-1, uvs-2, uvs-3, uvs-5 or uvs-6 show a differential effect on the two classes of ad-3 mutations.

PROJECT DESCRIPTION

OBJECTIVES: Previous studies (NIEHS-MB-003) have shown that the process of gene mutation is under genetic control and that both marked quantitative and qualitative differences can be found in such strains (when compared with the standard wild-type strain) with regard to mutation induction at specific loci. In two excision repair-deficient strains, upr-1 and uvs-2, enhanced mutant recovery was found after UV or x-irradiation as well as after treatment with various chemical mutagens. Genetic characterization of the UV-induced ad-3B mutants induced in the repair-deficient haploid strains showed that they were qualitatively different from the UV-induced ad-3B mutants in our standard wild-type strain (NIEHS-MB-003).

In a two-component heterokaryon, mutations in the ad-3 region result both from point mutation and chromosome deletions; the latter class is haplo-lethal and cannot be recovered in experiments on haploid strains. Various studies with variation in dose-rate with x-rays, photoreaction after UV, etc., have shown that the lesions giving rise to these two classes of mutations are qualitatively different. The objective of the present study is to determine the effect of upr-1, uvs-2, uvs-3, uvs-5 and uvs-6 on the induction of irradiation-induced chromosome deletions in the ad-3 region.

METHODS EMPLOYED: Both components of heterokaryon 12 (our standard wild-type strain) were crossed to various repair-deficient strains to enable us to construct new two-component heterokaryons which are homozygous for each mutation. These new strains (which are heterozygous for ad-3A and ad-3B) were then used in a series of x-ray and UV experiments to determine the effect of each repair-deficient mutation on spontaneous and radiation-induced mutation in the ad-3 region. In addition to upr-1 and uvs-2 heterokaryons from last year's work, new two-component heterokaryons have been developed which are homozygous for uvs-3, uvs-5 or uvs-6.

MAJOR FINDINGS: The results of mutation-induction experiments showed that spontaneous mutation in the ad-3 region in the new heterokaryons, homozygous for either upr-1 or uvs-2 was not different from heterokaryon 12. However, after x-irradiation, both strains required at least a 5- to 8-fold reduction in exposure to give similar forward-mutation frequencies as in the standard wild-type strain. The ad-3 mutants have been reserved for genetic analysis. In addition, after UV-irradiation, uvs-2 showed the same high sensitivity to inactivation and mutation-induction as the haploid uvs-2 strain. The ad-3 mutations also have been reserved for genetic analysis. Exploratory experiments have been started with the uvs-3, uvs-5 and uvs-6 strains using x-rays and UV; a satisfactory x-ray experiment has been obtained so far only with the uvs-5 heterokaryon.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recent studies by others have shown that in man xeroderma pigmentosa patients are deficient in excision repair. Cells of such patients in culture show enhanced inactivation after irradiation as well as enhanced induction of gene mutations and chromosome aberrations. Only limited analysis of the gene mutations can be made to determine whether they are qualitatively different from those found in experiments on normal human cells in culture. In Neurospora the ad-3

mutations recovered can be readily characterized by a series of simple genetic tests.

PROPOSED COURSE: Samples of ad-3 mutants induced by both x-rays and UV in all repair-deficient heterokaryons have been reserved for genetic analysis. This analysis will enable us to determine whether the enhanced recovery of ad-3 mutants in such strains is comparable for both point mutations and chromosome deletions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60002-04 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mutagenicity and Mutagenic Specificity of Environmental Agents in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS
OTHER:	F. J. de Serres	Branch Chief	EMB NIEHS

COOPERATING UNITS (if any)
Miles Laboratory, Elkhart, Indiana

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

Many synthetic nitrofurans and imidazole compounds are being used as chemotherapeutic agents or antibacterial food additives. Our previous studies with nitrofurans have shown that a chemotherapeutic agent, a food additive, an antischistosomal agent and an antitrichomoniasis agent are mutagenic in Neurospora crassa. Our studies also show that a NO_2 group at the C_5 position and a particular side chain at the C_2 position are necessary for the mutagenic activity of nitrofurans. It would be of interest to determine whether other chemotherapeutic agents are mutagenic in N. crassa, to verify function-structure relationship of nitrofurans and imidazole compounds and to determine the type of genetic alterations caused by chemotherapeutic agents.

The ad-3 test system of N. crassa developed by F. J. de Serres is used in this study. Conidia are treated with chemicals in pH 7 buffer at 30°C for 2 hours. At the end of the treatment chemicals are decanted and conidia are washed by centrifugation. The treated and untreated conidia are assayed for the presence of ad-3 mutants by the direct method. The isolated mutants are tested for genotype, complementation pattern and spectra of genetic alterations.

PROJECT DESCRIPTION

OBJECTIVES: Many synthetic nitrofurans and imidazole compounds are being used as chemotherapeutic agents in the treatment of human disease. Some others are being used as antibacterial food additives and preservatives. These compounds, therefore, are very closely related to human health. Our previous studies with nitrofurans have shown that a chemotherapeutic agent, a food additive and an antischistosomal agent are mutagenic in Neurospora crassa. Our studies also show that a NO_2 group at the C_5 position and a particular side chain at the C_2 position are necessary for the mutagenic activity of nitrofurans. It would be of interest to determine whether other chemotherapeutic agents are mutagenic in Neurospora crassa to verify function-structure relationships of nitrofurans and imidazole compounds and to determine the type of genetic alterations caused by chemotherapeutic agents. It would also be of interest to determine whether structurally related compounds cause similar types of genetic alterations.

METHODS EMPLOYED: The ad-3 test system of Neurospora crassa developed by F. J. de Serres is used in this study. In general, mutation induction experiments could be described as follows: conidia from a genetically marked two-component heterokaryon are harvested and are treated with different concentrations of nitrofurans in sodium-phosphate buffer, pH 7, at 30°C for two hours in a water bath shaker in the dark. At the end of the treatment chemicals are decanted and conidia are washed by centrifugation. The treated and untreated conidia are inoculated into 10 liters of Westergaard's medium with the appropriate supplements. After 7 days' incubation in the dark at 30°C, the frequency of purple ad-3 mutants are determined in each treatment. The isolated ad-3 mutants are tested for genotype, complementation pattern and spectra of genetic alterations at Miles Laboratory under a contract with the Institute.

MAJOR FINDINGS: In addition to 11 nitrofurans reported last year, the commonly used antischistosomal drug in China, F30066, was also found to be mutagenic in Neurospora. The mutagenicity of this compound, however, is less than AF-2, SQ18506, FANFT or H193.

The mutagenicity of three metronidazole related compounds has also been studied in N. crassa. These compounds (ZK Nr 26173, ZK Nr 25095 and ZK Nr 26306) are being considered as substitutes for metronidazole and to be used as chemotherapeutic agents for man. ZK Nr 26173 was found to be a potent mutagen while ZK Nr 25095 and ZK Nr 26306 are not mutagenic in Neurospora when conidia were treated. Whether the last two compounds are mutagenic in the growing vegetative cultures has not yet been studied.

Genetic analysis of ad-3 mutants induced by nitrofurans is still in progress. Available data indicate that AF-2, FANFT and SQ18506 induce predominantly base-pair substitution mutations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many nitrofurans are used as antibacterial drugs or as antibacterial food additives or food preservatives. Studies on the mutagenic activities of nitrofurans and imidazole compounds could provide information in regard to the potential mutagenic hazards of these agents in humans. Furthermore, studies on the mutagenicity and the chemical structure relationships of nitrofurans could lead

to a better understanding of the mutagenicity and other biological activities of nitrofurans such as antibacterial, antischistosomal and carcinogen activities and might lead to a production of non-mutagenic compounds that could be used as food additives or as drugs for the treatment of disease in man.

PROPOSED COURSE: Future studies will continue on those agents that are closely related to human health and those structurally related compounds. Future studies will also be carried out to determine types of genetic alterations caused by environmental agents and to determine the relationship between their mutagenic activities and their chemical structure.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60003-03 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mutagenic activity of chemical carcinogens in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS
	Frederick J. de Serres	Branch Chief	EMB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

Recent studies on the mutagenicity of chemical carcinogens in various systems have shown that there is a positive correlation between mutagenicity and carcinogenicity. Our previous studies with chemical carcinogens in Neurospora crassa not only show a positive correlation, but also indicate that potent chemical carcinogens induce predominantly base-pair substitution mutations. It is necessary to extend our studies to other carcinogenic compounds to determine whether those compounds are also mutagenic in Neurospora and to determine whether those compounds induce base-pair substitution mutations.

The ad-3 test system of N. crassa developed by de Serres is used in this study. Conidia are treated with chemical carcinogens. Treated and untreated conidia are assayed for the presence of ad-3 mutants by the direct method. The isolated mutants are analyzed by complementation, dikaryon and trikaryon tests.

PROJECT DESCRIPTION

OBJECTIVES: Recent studies on the mutagenicity of chemical carcinogens in various test systems have shown that there is a positive correlation between mutagenicity and carcinogenicity. Our previous studies in Neurospora crassa show that carcinogenic compounds in laboratory mammals are also mutagenic in N. crassa and that non-carcinogenic compounds are non-mutagenic. Our studies also indicate that potent carcinogens induce predominantly base-pair substitution mutations. It is necessary to extend our studies to other carcinogenic compounds to determine whether those compounds are also mutagenic in Neurospora and to determine whether those compounds induce base-pair substitution mutations. It is also necessary to extend our studies to the relationship between repair mechanisms and spectra of genetic alterations.

METHODS EMPLOYED: The ad-3 test system of N. crassa developed by F.J. de Serres is used in this study. Conidia from a genetically marked two-component heterokaryon are treated with chemical carcinogens. The treated and untreated conidia are analyzed for the presence of ad-3 mutants by the direct method. Based on the background counts and the number of mutants, the survival and the mutation frequency are determined. The ad-3 mutants isolated from forward mutation experiments are made homokaryotic for the adenine requirements. The genotype and the complementation pattern of each mutant is determined by heterokaryon tests with various strains used as testers. Mutants are then tested by means of dikaryon and trikaryon tests to determine the frequency of chromosomal deletions.

MAJOR FINDINGS: Genetic analysis of ad-3 mutants induced by chemical carcinogens show that potent chemical carcinogens such as 4NQO, 4HAQO, PMMT, aflatoxin B_1, 2AN and FANFT induce predominantly base-pair substitution mutations in Neurospora. Two epoxy carcinogens, DEP, and DEO, appear to cause similar spectra of point mutations. DEO, however, induces a high frequency of chromosomal deletions. Measurement of molecular distances show that DEO, but not DEP, can cause interstrand DNA cross linkage.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of chemical carcinogens in N. crassa might provide a system that could be used for screening carcinogenic chemical agents in the human environment. Furthermore, the studies on the spectrum of genetic alterations, caused by chemical carcinogens, both in wild-type and repair-deficient strains of N. crassa might provide information in regard to the relationship between carcinogenesis and mutagenesis of chemical agents.

PROPOSED COURSE: Future studies on the mutagenic activity of chemical carcinogens will mainly be carried out under the contract with Dr. Herman E. Brockman at Illinois State University. The mutagenicity and the mutagenic specificity of chemical carcinogens will be studied both in a wild-type and repair-deficient strain of N. crassa.

PUBLICATIONS

Ong, T. and Malling, H.V.: Microsomal activation of dimethylnitrosamine to metabolites mutagenic in Neurospora crassa. Mutation Research 31:195-196,1975.

Ong, T. and de Serres, F.J. Mutation induction by difunctional alkylating agents in Neurospora crassa. Genetics 80:475-482, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60004-03 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Development of a spot test system in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS
OTHER:	Janet Guthrie	Microbiologist	EMB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

SUMMARY OF WORK (200 words or less - underline keywords)

Development of a spot test will provide a simple, economical and quick system in eukaryotes for the detection and evaluation of the mutagenic activity of chemical carcinogens and environmental agents.

We have established optimal conditions for the spot test and have selected four strains from hundreds of mutants as tester strains. Conidia from 7-day-old vegetative cultures of testers are harvested and 2 x 10^7 conidia in 1 ml saline are added into 2 ml of cover medium The mixture of conidia and cover medium is poured onto a plate containing 20 ml of Fries' minimal medium. Samples of an environmental agent are placed on the center of the plate. After 7 days' incubation at 30°C, revertants from each plate are recorded. The results of our studies show that the spot test system of Neurospora can be used for screening the mutagenicity of environmental agents. We are in the process of improving our spot test system by introducing UV sensitive markers into tester strains. We are also in the process of developing a forward mutation spot test system.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The adenine-3 (ad-3) forward mutation test system in Neurospora crassa developed by F. J. de Serres is one of the best systems for studying chemical and physical mutagenesis in eukaryotic microorganisms. With this test system, recessive lethal mutations resulting from either chromosome deletions or point mutations can be recovered at the ad-3 region. The isolated mutants can be characterized by a series of genetic tests and the type of genetic alterations at the molecular level can be determined. The limitations of this test system, however, are that one cannot screen for the mutagenicity of a large number of chemicals in a short period of time and that not every laboratory can use this test system. It seems, therefore, necessary to develop a spot test and suspension test system in N. crassa so that both reverse and forward mutations caused by environmental agents can easily be detected with simple test systems.

METHODS EMPLOYED: Conidia from 7-day-old vegetative cultures of ad-3A testers are harvested and 2 x 10^7 conidia in one ml of saline are added into 2 ml of cover medium (Fries' minimal medium). The mixture of conidia and cover medium is poured onto a plate containing 20 ml of Fries' minimal medium. Samples of environmental agents or mutagens are placed on the center of the plate. After 7 days' incubation at 30°C, revertants from each plate are recorded.

In order to increase their sensitivity, testers are being crossed to UV sensitive mutants. Mutants carrying both ad-3A and UV-sensitive markers are being selected. The sensitivity of these isolates to chemicals will be tested with a spot test.

For the development of a forward mutation spot test, the lowest concentrations of 5-fluorodeoxyuridine (5F) and cyclohexamide (CH) which will inhibit the growth of Neurospora conidia are being determined by plating conidia in the medium containing these compounds. Conidia from tester strains will be treated with mutagens and will be plated in the medium containing 5F or CH. CH or 5F resistants will be scored after plates are incubated for 7 days.

MAJOR FINDINGS: We have established optimal conditions for the spot test and have selected 4 strains from hundreds of mutants as tester strains. The mutagenicity of approximately 50 compounds has been tested with a spot test. The results are comparable to those of forward mutation experiments. These results suggest that the spot test system of N. crassa can be used for screening the mutagenicity of environmental agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of a spot test system will provide a simple, economical and quick system in eukaryotes for the detection and evaluation of the mutagenic activity of chemical agents in the human environment.

PROPOSED COURSE: Future efforts will be (1) to increase the sensitivity of tester strains by introducing UV sensitive markers into these testers, (2) to incorporate an in vitro activation technique into spot and suspension test systems and (3) to develop a forward mutation spot test or suspension test system in Neurospora.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60005-03 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mutagenic activity of antischistosomal drugs and their analogs in Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS
OTHER:	Frederick J. de Serres	Branch Chief	EMB NIEHS

COOPERATING UNITS (if any)
Miles Laboratory, Elkhart, Indiana

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

Hycanthone, lucanthone and niridazole, chemotherapeutic agents in the treatment of schistosomiasis, are attracting wide concern because of their potential carcinogenic and mutagenic hazards to treated individuals in human population. Our studies have shown that hycanthone, lucanthone and their indazole analogs and niridazole are mutagenic in Neurospora crassa. It would be of interest to determine whether hycanthone induces particular types of genetic alterations and whether structurally related compounds cause similar spectra of genetic changes.

The genotype and the complementation patterns of each mutant are determined by growing each mutant with each complementation tester. The frequencies of chromosomal deletions are determined by dikaryon and trikaryon tests.

Genetic analysis of ad-3 mutants induced by hycanthone and its analogs indicates that these compounds cause similar spectra of genetic alterations in which multilocus deletion is a predominant type.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: Hycanthone, lucanthone and niridazole, chemotherapeutic agents in the treatment of schistosomiasis, are attracting wide concern because of their potential carcinogenic and mutagenic hazards to treated individuals in the human population. Our previous studies have shown that hycanthone, lucanthone and their indazole analogs are mutagenic in Neurospora crassa. Niridazole is not mutagenic under the condition that conidia were treated. This compound is mutagenic, however, when growing vegetative cultures of N. crassa were treated. It would be of interest to determine whether hycanthone induces particular types of genetic alterations and whether structurally related compounds cause similar spectra of genetic changes.

METHODS EMPLOYED: The ad-3 mutants isolated from forward mutation experiments with hycanthone, lucanthone and their indazole analogs and niridazole in Neurospora are sent to Miles Laboratory for genetic analysis These mutants are made homokaryotic for the adenine requirements. The genotype and the complementation patterns of each mutant are determined by growing each mutant with each complementation tester. Based on the complementation patterns, mutants can be classified as non-complementing, polarized complementing and non-polarized complementing mutants. The mutants are further tested with dikaryon and trikaryon tests to determine the frequency of chromosomal deletions.

MAJOR FINDINGS: The mutagenic activities of hycanthone, lucanthone and their indazole analogs in N. crassa indicate that compounds with a methyl group at the C_4 position are more mutagenic than compounds with a methanol group; 6-chloro-indazole analogs are more mutagenic than nonchlorinated analogs. The data from N. crassa show that there is no correlation between mutagenicity and antischistosomal activity.

The results of genetic analysis of ad-3 mutants induced by hycanthone and its analogs indicate that these compounds cause similar spectra of genetic alterations in which multilocus deletion are a predominant type.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of niridazole, hycanthone, lucanthone and their structural analogs are essential and important, since hycanthone and niridazole are widely used for treatment of schistosomiasis in the human population. It is important to determine the potential mutagenic hazards of these compounds to the treated individuals. Studies on the relationship between structures and mutagenic activities of hycanthone and its analogs might lead to a production of non-mutagenic compounds that can be used for the treatment of schistosomiasis. This analysis will provide some experience with regard to structure-function relationships which will be important for future work with mutagenic environmental chemicals. We need to know whether we can develop structural analogs of desirable, but mutagenic chemicals that will retain the desired pharmacological properties, but not the undesirable mutagenic properties.

PROPOSED COURSE: This project will be determined after all mutants are genetically analyzed. The results of mutants characterization will be published in the near future.

PUBLICATIONS

Ong, T. and de Serres, F.J.: Mutagenic evaluation of antischistosomal drugs and their derivatives in Neurospora crassa. J. Toxicol. Envir. Health 1:271-279, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60006-02 EMB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mutagenicity and mutagenic specificity of chemical carcinogens in UV-sensitive strains of Neurospora crassa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hirokazu Inoue	Visiting Fellow	EMB NIEHS
OTHER:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS
	Frederick J. de Serres	Branch Chief	EMB NIEHS

COOPERATING UNITS (if any)

Miles Laboratory, Elkhart, Indiana

LAB/BRANCH

Environmental Mutagenesis Branch

SECTION

Microbial Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

Many studies, in bacteria as well as in other microbial organisms, have shown that some of the repair-deficient strains are more mutable than the wild type. In studies with the ad-3 test system of Neurospora crassa, F. J. de Serres and his co-workers showed that repair-deficient strains, upr-1 and uvs-2, are more mutable than wild type after UV or gamma-ray irradiations. It has also been shown by de Serres that the spectra of genetic alterations caused by UV in repair-deficient strains are different from that caused by UV in the wild type. It is of interest to study and compare the mutagenic sensitivity of repair-deficient and wild-type strains to various chemical carcinogens and to compare the spectra of genetic alterations caused by these carcinogenic agents in the UV-sensitive and the wild-type strains.

PROJECT DESCRIPTION

OBJECTIVES: Many studies, in bacteria as well as in other microbial organisms, have shown that some of the repair-deficient strains are more mutable than the wild type. In studies with the ad-3 test system of Neurospora crassa, F. J. de Serres and his co-workers showed that the repair-deficient strains, upr-1 and uvs-2, are more mutable than wild type after UV or gamma-ray irradiations. It has also been shown by de Serres that the spectra of genetic alterations caused by UV in repair-deficient strains are different from that caused by UV in the wild type. It is of interest, therefore, to study and compare the mutagenic sensitivity of repair-deficient and wild-type strains to various chemical carcinogens and to compare the spectra of genetic alterations caused by these carcinogenic agents in the UV-sensitive and the wild-type strains.

METHODS EMPLOYED: The ad-3 test system of Neurospora crassa developed by F. J. de Serres is used in this study. Conidia from UV-sensitive strains or wild type are treated with chemical carcinogens. The treated and untreated conidia are analyzed for the presence of ad-3 mutants by the direct method. The isolated ad-3 mutants from forward mutation experiments are made homokaryotic for the adenine requirement. The genotype and complementation pattern are determined by complementation tests.

MAJOR FINDINGS: Results of mutation induction experiments with ICR-170 indicate that upr-1, uvs-6 and wild type strains have a similar mutagenic response, while uvs-2 and uvs-5 are both three times and uvs-3 is five times less mutable than wild-type strain. Therefore, the relative mutagenic responses of the UV-sensitive strains and the wild type to ICR-170 are different from the relative response observed with UV, x-rays and MNNG.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study shows that the repair-deficient strains, upr-1 and uvs-2, are not only more mutable than wild type by UV or gamma-ray irradiation but also more sensitive to the chemical carcinogen, MNNG. If these strains are also more sensitive than wild type to other chemicals, the use of repair-deficient strains would facilitate the detection of mutagenic activity in various environmental agents.

PROPOSED COURSE: Studies on the mutagenicity and mutagenic specificity with other carcinogens will be performed in these repair-deficient strains and the role of repair ability in mutagenesis and carcinogenesis will be investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60007-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Relationship Between Self-Inhibitors of Germination and Mutation Induction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barry R. Scott	Visiting Fellow	EMB NIEHS
OTHER:	Laila Moustafa	Senior Staff Fellow	ETB NIEHS
	John Elliott	Postdoctoral Fellow	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

SUMMARY OF WORK (200 words or less - underline keywords)

Recent studies with Aspergillus have shown that marked differences in induced mutation frequencies after apparently replicate mutagenic exposure are mainly due to the presence of varied amounts of self-inhibitors of germination in the conidia at the time of mutagenic treatment. With some mutagens, removal of these compounds has also affected the qualitative mutational response of thirteen genes which may be simultaneously studied in this organism, Aspergillus nidulans. It would be very interesting to determine whether such substances are also present in the conidia of Neurospora crassa and to investigate the effect these substances might have on the processes of mutation induction.

Such a program, it is hoped, will increase the confidence with which the mutagenic data obtained with fungal test systems can be meaningfully extrapolated to man. Isolation of inhibitory substances might also be very useful in extending the presumptive mutagenic treatment times which may be given to fungi in a host-mediated assay or microsomal activation situation, hence increasing the detection capability of this organism.

PROJECT DESCRIPTION

OBJECTIVES: These are (1) to determine whether inhibitor substances for germination are present in Neurospora crassa conidia, (2) to establish conditions under which quantitative results may be obtained with Aspergillus and Neurospora, (3) to isolate and identify these substances in Aspergillus and Neurospora, (4) to investigate the effect of the presence these substances have on mutation induction and (5) to evaluate the value of these compounds to extend the treatment times which may be given to fungi in a host-mediated or microsomal activation system.

METHODS EMPLOYED: Routine microbial and biochemical techniques are being employed in this investigation. In the future it is proposed that the 2-thioxanthine and methionine suppressor mutation screening systems of Aspergillus, which detect forward mutation at about 22 gene loci simultaneously plus the heterokaryon 12 system of Neurospora, are used to investigate the effect these compounds have on mutation induction.

MAJOR FINDINGS: This investigation has determined that a self-inhibitor of germination exists in Neurospora crassa. Removal of these compounds from the conidia of this organism, as previously shown in Aspergillus nidulans, is essential in order to obtain quantitative results after treatment with deleterious agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: One of the greatest difficulties facing the establishment of test systems for routine quantitative mutagenicity testing is the heterogeniety of the test organism. Any procedure which reduces this biological heterogeniety increases the confidence with which the mutagenic data obtained with a test system can be meaningfully extrapolated to man. By controlling the amount of possible germination inhibitory substances in Neurospora and Aspergillus conidia, the degree of heterogeniety of any mutagenic test system which uses these organisms is decreased. Isolation of the inhibitory substance might also be very useful in extending the treatment time which may be given to fungi in a host-mediated assay or microsomal activation situation, hence increasing the detection capability of the fungi. Finally, such a study should also lead to a greater understanding of the basic processes involved in mutation induction.

PROPOSED COURSE: Studies will now be directed toward detection and isolation of the possible self-inhibitory substances in the conidia of Aspergillus and Neurospora. Then the effect of these substances on mutation induction will be investigated.

PUBLICATIONS

Scott, Barry R.: The effect of various "Magdala Reds" in inducing paramorphic colony growth in Neurospora crassa. (in preparation)

Scott, Barry R.: The presence of a germination inhibitor in Neurospora crassa and its effect on mutagen sensitivity (in preparation).

Scott, Barry R. and Moustaffa, Laila A.: An observation on conidial germination lines of Neurospora crassa. (in preparation).

Scott, Barry R. and Moustaffa, Laila A.: The germination of diethyl ether prepared conidia of Neurospora crassa. (in preparation). –

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60008-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mutagenic Activity of Furocoumarins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barry R. Scott	Visiting Fellow	EMB NIEHS
OTHER:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS
	William Sheridan	Section Head	EMB NIEHS
	Eugene Soares	Senior Staff Fellow	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

SUMMARY OF WORK (200 words or less - underline keywords)

Furocoumarins in combination with blacklight are being used as chemotherapeutic agents in the treatment of diseases in man such as Leukoderma and Psoriasis. These compounds are also commonly occurring in man's natural and artificial environment. The possible exposure of the human population to these compounds is, therefore, relatively high and cause for concern. Systematically, little information is known about the comparative mutagenic, carcinogenic and teratogenic effects of the furocoumarins. It would be interesting to determine the comparative mutagenicity of one of the furocoumarins, 8-methoxypsoralen (8-MOP) with various combinations of blacklight in a variety of microbial systems and to determine the type of genetic alteration caused by 8-MOP in each region. In the later stages of the project, the investigation will be expanded to other furocoumarins in order to determine whether there is a relationship between the mutagenicity and chemical structure of the furocoumarins.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: These are (1) to determine the comparative mutagenicity of one of the furocoumarins, 8-methoxypsoralen (8-MOP), with various combinations of blacklight in a variety of microbial systems, (2) to determine the type of genetic alteration caused by 8-MOP in each regime. (3) In later stages of the project the investigation will be extended to other furocoumarins in order to determine whether there is a relationship between the mutagenicity and chemical structure of the furocoumarins. (4) Evolution of rapid screening systems to detect photosensitizers in microbes and possibly mice.

METHODS EMPLOYED: Standard quantitative screening systems which detect mutagens are being employed in the first part of the investigation.

MAJOR FINDINGS: In the absence of radiation these compounds do not appear to induce mutations in Salmonella typhimurium, Escherichia coli and Aspergillus nidulans. However, in combination with radiation 8-MOP is a potent mutagen, while under normal testing procedures effects of these compounds would be detected.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Studies on the mutagenicity of the furocoumarins are essential and important because some of the compounds are widely used for treatment of Leukoderma and Psoriasis in human populations. The relationship between various microbial test systems could provide information on the potential mutagenic hazard of 8-MOP in humans. Furthermore, studies on the mutagenicity and the chemical structure of the furocoumarins could lead to a better understanding of the mutagenicity and other biological activities of the furocoumarins; this might result in either the production of non-mutagenic compounds that could be used for the treatment of diseases in man or treatment conditions which would reduce the exposure risk.

The reason for expanding this study into one, aimed at establishing screening systems in microbes and mice, is that it is common knowledge in pediatrics that newborn infants are transparent to visible light due to the fact that they lack pigmentation. As a matter of fact, physical examination using transillumination is very common in neonatology. Hence, any undetected photosensitizing drug administered to these infants (or their breast-feeding mothers?) could result in an increased genetic risk in future populations.

PROPOSED COURSE: Studies with various microbial test systems which detect mutation induction will continue to be used in assessing three major types of treatment condition for mutagenesis. These are (a) 8-MOP without radiation, (b) 8-MOP plus low doses of radiation and (c) 8-MOP plus high doses of radiation. Results obtained from these experiments will then be used as a basis to establish rapid screening systems in microbes and mice. Future studies will also be carried out to determine the types of genetic alterations caused by furocoumarins as well as determining the relationship between mutagenic activity and chemical structure.

PUBLICATIONS

Scott, Barry R.: Failure to detect a mutagenic activity of 8-methoxypsoralen (in the dark) in strains of Salmonella typhimurium and Escherichia coli. Mutation Research (in press).

Scott, Barry R., Pathak, Madhu A. and Mohn, Georges R.: Molecular and genetic basis of furocoumarin reactions. Reviews in Genetic Toxicology (in preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60012-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Interaction of Heavy Metal Ions with Synthetic DNA Polymers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael W. Lieberman	Section Head	EMB NIEHS
	David Amacher	Postdoctoral Fellow	EMB NIEHS
OTHER:	Donald Harvan	Chemist	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

We have studied the interactions of a series of heavy metal ions with synthetic DNA polymers to determine the effects of these ions on buoyant density in the analytical ultracentrifuge. This has enabled us to determine what types of sequences react preferentially with these ions and to determine how they might be most useful in separating natural DNA's of known sequence from one another. In addition, based on these data, one may make some logical predictions about the relative mutagenicity of these agents for loci containing runs of particular nucleotides. Thus, these results should be of special interest to geneticists interested in metal ion mutagenesis.

PHS-6040

PROJECT DESCRIPTION

OBJECTIVES: It is now widely appreciated that heavy metals may have deleterious effects on living organisms. Because of the variety of the metals involved and the diversity of the effects, it is likely that a number of different mechanisms are involved. Recent studies have indicated that some heavy metals are mutagenic and they may form physical complexes with DNA and chromatin. To learn more about the interaction of metal ions with DNA, we have begun by studying the effect of Hg^{2+} and Ag^{+} on the buoyant density of synthetic DNA polymers of defined sequence. In addition, we are studying the effects of chloroplatinic acid, cis-diaminodichloroplatinium and trans-diaminodichloroplatinium using similar methods. It is hoped that eventually such studies will be useful in elucidating the mechanism by which genetic alterations occur.

METHODS EMPLOYED: Analytical and preparative density equilibrium centrifugation in Cs_2SO_4; spectrophotometric thermal denaturation studies; thin-layer chromatography.

MAJOR FINDINGS: When Hg^{2+} or Ag^{+} are complexed with synthetic DNA polymers of different base sequence, a variety of interacting changes are noted: Hg^{2+} results in a large increase in the buoyant density of poly(dA-dT), but has little effect on the densities of poly(dA)·poly(dT), poly(dG-dC), or poly(dG)·poly(dC). Hg^{2+} causes strand separation of poly(dA)·poly(dT), probably by binding extensively to intrastrand thymines. Ag^{+}, on the other hand, induces a large change in the buoyant density of poly(dG-dC) and has a lesser effect on other polymers. The two diaminoplatinium compounds have marked effects on the buoyant densities of these polymers while chloroplatinic acid is inactive. These results imply a selectivity of binding of these metal ions to DNA sequences which is based, at least in part, on nearest neighbor sequence.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These findings have a number of implications for the types of genes which are mutable by metal ions: Ag^{+}, for instance, attacks poly(dG-dC) sequences much more readily than poly(dG)·poly(dC). As a consequence, it may be more mutagenic for sequences rich in GCGCGC stretches than for ones rich in GGGGG sequences. In addition, these findings allow us to explain much of the heretofore unexplained behavior of naturally occurring DNA sequences (satellite DNAs, 5s DNA, rDNA) in metal ion - Cs_2SO_4 gradients and to diverse methodology for isolation of specific genes. The data with the platinium compounds may help explain some of the biological effects of these agents.

PROPOSED COURSE: This work is in its final phases and will be completed soon.

PUBLICATIONS

Lieberman, M.W., Harvan, D.J., Amacher, D.E. and Patterson, J.B.: Effect of Mercuric and Silver Ions on Cesium Sulfate Equilibrium Buoyant Densities of Synthetic Polydeoxyribonucleotides. Biochim. Biophys. Acta: 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60013-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Interaction of Mutagens and Carcinogens with DNA and Polydeoxyribonucleotides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald P. Harvan	Chemist	EMB NIEHS
	Michael W. Lieberman	Section Head	EMB NIEHS
OTHER:	Ronald Hass		EBCB NIEHS

COOPERATING UNITS (if any)
Environmental Biology and Chemistry Branch

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

Many investigators have observed that mutagens and carcinogens may be covalently bound to DNA; however, no investigations to our knowledge have looked at the effect of base sequence on binding. Thus, we compared the amount of binding of the direct acting carcinogen N acetoxy-2-aminofluorene with four synthetic double-stranded polymers of known sequence. At extents of reaction of one percent or greater, poly(dG-dC) was attacked 2-3 times as readily as poly(dG)·poly(dC) and both were attacked much more readily than polymers containing AT. At very low extensive reaction the attack on the two GC containing polymers was about equal, suggesting that at higher levels of attack there was some positive interaction between the first molecule bound and additional attacking carcinogen molecules.

PROJECT DESCRIPTION

OBJECTIVES: One of the most exciting developments in the field of chemical carcinogenesis is the observation that many chemical carcinogens are mutagens. Although previous studies have demonstrated that direct-acting carcinogens bind covalently to DNA, relatively little work has been done on the type of adducts formed and especially on the role of base sequence and nearest neighbors on binding. Such information would be of great use in the understanding of the mutagenic effects of carcinogens and in helping to identify regions of the genome which might be hypersensitive to chemical attack. We have begun such studies by looking at the binding of a model carcinogen (N-acetoxy-2-acetylaminofluorene) to four synthetic polymers: poly(dA-dT); poly(dA)·poly(dT); poly(dG-dC) and poly(dG) poly(dC).

METHODS EMPLOYED: Enzymatic digestion studies; thin-layer and column chromatography; mass spectroscopy.

MAJOR FINDINGS: At relatively high levels of polymer substitution (1-10%), poly(dG-dC) is attacked about twice as readily as poly(dG)·poly(dC). Binding to A&T containing polymers is about an order of magnitude less. At low levels of binding (0.5%), the attack on the two GC containing polymers is about equal and still much greater than that to AT containing polymers. Mass spectroscopy and NMR data suggest that the major product is a C^8 guanine adduct.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Definition of the products of reaction between carcinogens and/or mutagens and DNA is a pre-requisite for any comprehensive understanding of the molecular basis of chemical mutagenesis. Thus, these studies are a step in this direction.

PROPOSED COURSE: To carry out the above outlined research and eventually begin to examine other selected chemical mutagens and naturally occurring DNAs of defined sequence.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60014-02 EMB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Removal of Covalently Bound Mutagens and Carcinogens from the DNA of Cultured Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David E. Amacher	Postdoctoral Fellow	EMB NIEHS
	John A. Elliott	Postdoctoral Fellow	EMB NIEHS
OTHER:	Michael W. Lieberman	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Mutagenesis Branch

SECTION

Somatic Cell Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

SUMMARY OF WORK (200 words or less - underline keywords)

It has been known for several years that mammalian cells remove covalently bound mutagens and carcinogens from their DNA; however, few comparative quantitative studies have been attempted. We have begun such work by analyzing the removal of adducts of N acetoxy-2-aminofluorene in a variety of mammalian and human cells. To date, it is clear that somewhere between 20 and 50 percent of the carcinogen may be removed by G_1 cells in culture, but the overall evaluation of the kinetics of removal are not yet available.

PROJECT DESCRIPTION

OBJECTIVES: A variety of mammalian cells initiate DNA repair synthesis in response to damage by chemical mutagens and carcinogens. Usually this process is studied by following the incorporation of radioactive precursors into non-replicating DNA. Much less work has been done on the removal of bound carcinogen; however, analysis of the removal of covalently bound mutagens and carcinogens is of great importance since this approach allows quantitation of the amount of damage actually removed during repair. To help assess the importance of repair synthesis in mutagenesis and carcinogenesis, we have begun to examine the removal of bound acetylaminofluorene (a potent chemical carcinogen and mutagen) and thymine dimers produced by ultraviolet radiation from a number of mammalian cell lines. Such studies should allow quantitative estimation of the extent of removal bound carcinogen and rates of removal of different adducts in different cell lines.

METHODS EMPLOYED: Isotopic-labeling, CsCl isopycnic centrifugation, cell culture.

MAJOR FINDINGS: All cell types examined thus far, including mouse cells (Balb/c, 3T3), guinea pig embryo cells, rat kangaroo cells and human diploid fibroblasts (WI-38), remove substantial bound acetylaminofluorene (30-60%) in 24 hours. Surprisingly, mouse cells which are supposedly less repair-competent than human cells remove more bound carcinogen than human cells. At present we are comparing the rates of removal of bound carcinogen and thymine dimers in the different cell lines including xeroderma pigmentosum cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is hoped that these studies will provide a clearer understanding of the role of repair in modifying mutagenesis and perhaps carcinogenesis. Our initial studies indicate that assessment of repair competence on the basis of thymine dimer removal alone may be misleading, especially for evaluation of processes related to mutagenesis and carcinogenesis.

PROPOSED COURSE: The project is in its early stages and will be continued along the same lines.

PUBLICATIONS

Lieberman, M.W., Amacher, D.E., Elliott, J.A. and Huang, S.L.: Removal of Bound Carcinogen from the DNA of Mammalian Cells in Culture. GANN 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60015-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Analysis of the Effects of Mutagens and Carcinogens on Nuclear Protein Synthesis in Cultured Human Diploid Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Michael W. Lieberman Section Head EMB NIEHS
Gary S. Stein*

COOPERATING UNITS (if any)
*Department of Biochemistry, University of Florida, School of Medicine, Gainesville, Florida

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

We have looked at the synthesis of histones and non-histone nuclear proteins during DNA repair synthesis in human cells damaged with ultraviolet radiation and a powerful chemical mutagen and carcinogen. It is of interest that although damage to these cells with ultraviolet radiation and the carcinogen produces up to a fifty-fold increase in DNA synthesis (DNA repair synthesis), no new accumulation of histones or non-histone nuclear proteins was noted in these cells. Thus, although large chunks of DNA (in the range of 100-140 nucleotides) are removed during excision and subsequent repair, there is no evidence that new histones or non-histone nuclear protein synthesis need accompany this process. It appears that either nuclear proteins remain in place during repair synthesis or that they are removed and then subsequently re-utilized.

PROJECT DESCRIPTION

OBJECTIVES: Cultured human cells repair DNA damage induced by ultraviolet radiation and chemical mutagens and carcinogens. Little is known, however, about the fate of DNA-associated proteins (histones and non-histone nuclear proteins) during repair synthesis. An understanding of the metabolism of these proteins is of importance since rearrangements or derangements of their structure or function could lead to alterations in gene expression or activation with important cellular consequences. We have begun an investigation in this area by examining the rates of synthesis of histones and acidic nuclear proteins during repair synthesis.

METHODS EMPLOYED: Cell culture; isotopic labeling; CsCl equilibrium centrifugation; gel electrophoresis; liquid scintillation counting.

MAJOR FINDINGS: Human cells repair damage to DNA induced by ultraviolet radiation and N-acetoxy-2-acetylaminofluorene (NA-AAF) without the synthesis of new histones or acidic nuclear proteins.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since about 100 bases (10 turns of the double helix) are removed during repair of NA-AAF or UV damage, the above result suggests either that damage occurs primarily in stretches not covered by nuclear proteins or (more likely) that nuclear proteins are reutilized (conserved) during repair synthesis.

Alternatively, regions in which repair has occurred remain naked. Distinguishing among these models is of great importance since it may allow one to examine how mutagenesis and gene expression interact.

PROPOSED COURSE: We propose to devise experiments to distinguish among these possibilities, to analyze the distribution of damage induced by these agents and to begin studies of nuclear protein alteration (phosphorylation, acetylation, methylations, etc.).

PUBLICATIONS

Stein, G.S., Park, W.D., Stein, J.S. and Lieberman, M.W.: Synthesis of Nuclear Proteins during DNA Repair Synthesis in Human Diploid Fibroblasts Damaged with Ultraviolet Radiation or N acetoxy-2-acetylaminofluorene. Proc. Nat. Acad. Sci. 73: 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60016-02 EMB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

In Vitro Transformation and Mutagenesis in Cultured Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.A. Elliott	Postdoctoral Fellow	EMB NIEHS
	S.L. Huang	Staff Fellow	EMB NIEHS
	Y. Ishii	Visiting Fellow	EMB NIEHS
OTHER:	M.W. Lieberman	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Mutagenesis Branch

SECTION

Somatic Cell Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

SUMMARY OF WORK (200 words or less - underline keywords)

We have begun a series of experiments to measure in vitro transformation and mutagenesis simultaneously in cultured mammalian cells exposed to mutagens and carcinogens. Thus far we have been able to develop a plaque assay which consists of treating a subclone of this line with carcinogens and mutagens and plating them directly into soft agar. The major advantage of this technique (which is only possible with certain types of cultured cells) is that one may measure transformation frequency directly without first plating in liquid medium, retrypsinizing and then plating in soft agar. Thus quantitation is much more accurate. To date we have been able to compare the transformational effects of three carcinogens and ultraviolet radiation. We are still developing a mutagenesis system which we can use in conjunction with the transformation assay; most likely this will be a variation of hypoxanthine-guanine phosphoribosyl transferase system. Within the next six months we should be able to make quantitative comparisons between transformation in vitro and mutagenesis.

PROJECT DESCRIPTION

OBJECTIVES: Many recent studies have demonstrated that carcinogens act as mutagens in microbial test systems and cultured mammalian cells. It is unclear, however, whether the mutagenic action of carcinogens is related to their oncogenic potential or is an unrelated phenomenon. Since carcinogens have a wide variety of other effects on living cells including alterations in DNA, RNA and protein synthesis, membrane changes, changes in gene expression and toxic effects, it would be of interest to begin to evaluate the role of these events and mutagenesis in the oncogenic process. We have begun to examine mutagenesis and in vitro transformation simultaneously in the same cell lines and to evaluate the dosimetry of these processes using isotopically labeled carcinogens. The objective is to help clarify what role, if any, mutagenesis plays in transformation and carcinogenesis.

METHODS EMPLOYED: Cell culture, mutagenesis assay (HGPRT locus), transformation assay (growth in soft agar), radioisotope techniques, CsCl isopycnic centrifugation, thin-layer chromatography.

MAJOR FINDINGS: We have developed a system to quantitate transformation by chemicals with a simple "plaque assay" (soft agar) technique. Thus far on a concentration basis 4-nitroquinoline-1-oxide is more active than N-acetoxy-2-acetylaminofluorene which is more active than MNNG. Ultraviolet radiation also transforms cells in this system. A number of the transformed lines produce tumors in hamsters.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Hopefully this project should help clarify the relationship between mutagenesis and carcinogenesis.

PROPOSED COURSE: Proceed as indicated above.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60017-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mutation Induction in Human Fibroblasts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shiu Lan Huang	Senior Staff Fellow	EMB NIEHS
OTHER:	Michael W. Lieberman	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

A system to detect mutations at the HGPRT locus in cultured, diploid human fibroblasts has been established in this laboratory. The carcinogen N-acetoxy-2-acetoaminofluorene has been studied and found to be mutagenic in human cells. Dose response in the induced mutation frequency was demonstrated. As judged by enzyme assays, most of the cells which grow in selective medium are true mutants.

PROJECT DESCRIPTION

OBJECTIVES: A system to detect somatic cell mutation as measured by resistance to guanine analogs has been established using cultured, diploid human fibroblasts both in this and other laboratories. Because of the relevance of the cultured diploid human fibroblast and because so much is known about the biochemistry and genetics of the HGPRT locus (HGPRT = hypoxanthine guanine phosphoribosyl transferase), this mutation detection system is potentially useful for environmental monitoring.

METHODS EMPLOYED: The simplest explanation is that thioguanine or azaguanine (TG and AG) resistance results from a deficiency of the enzyme HPRT which is specified by a gene on the X chromosome. A million human skin fibroblasts were treated with NA-AAF for one hour. The treated cells were dispersed and 2.5×10^4 cells were inoculated in petri dishes. On day 4, selection using 3×10^{-5}M TG was initiated. The selective medium was renewed twice a week. The cultures were stained and TG^r (thioguanine resistant) colonies were counted or isolated on day 29. TG^r colonies were then propagated and the HGPRT activity was determined. The ability to use purine salvage pathways was also investigated for all the TG^r lines by growing them in azaserine and hypoxanthine.

MAJOR FINDINGS: The mutagenicity of NA-AAF has been studied using the TG resistance testing system. Four doses, 10^{-5}, 7.5×10^{-6}, 5×10^{-6} and OM NA-AAF, were used. NA-AAF was mutagenic to human fibroblast. A dose-response relationship for induced mutation frequency using NA-AAF was demonstrated. All the TG^r colonies obtained are true mutants (HPRT-deficient) and the resistant phenotype was maintained after several divisions in the absence of thioguanine. Thus, the change is stable.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The Lesch-Nyhan syndrome is a heritable disorder of purine metabolism in man. The responsible gene is sex-linked recessive. The primary product of the gene is the enzyme hypoxanthine guanine phosphoribosyl transferase which is defective or absent in this syndrome.

With knowledge of this inherited genetic defect, a system using this locus can be established to monitor the induction of mutations by environmental agents.

PROPOSED COURSE: We propose to characterize this system further with regard to other agents and the nature of the genetic lesions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60018-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Isolation of Human Ribosomal Cistrons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Michael W. Lieberman Section Head EMB NIEHS
Darrel Stafford*

COOPERATING UNITS (if any)
*Department of Zoology, University of North Carolina, Chapel Hill, NC

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

SUMMARY OF WORK (200 words or less - underline keywords)

We have been attempting to isolate the DNA sequences which code for ribosomal RNA from human material. It is the goal of this work to determine how the sequences are organized in the nucleus of human cells and to provide purified isolated human genes which may be studied for their interaction with specific mutagens and carcinogens. It is hoped that by providing isolated pure genes of known sequence that the molecular events of carcinogen DNA interactions may be studied in fine detail. To date our preparations are only partially pure, but it is hoped that within the next six months to a year, we will have considerable quantities of isolated human rDNA sequences.

PHS-6040

PROJECT DESCRIPTION

OBJECTIVES: It is clear that mutation cannot be understood in molecular terms without a detailed knowledge of the base sequence and structure of the genes involved. A pre-requisite for this type of analysis is physical isolation of the genes of interest. Our initial effort has been directed toward the isolation of the human ribosomal cistrons (the genes which code for ribosomal RNA). Isolation and characterization of these cistrons should provide a substrate for analyzing the interaction of these genes with chemical mutagens and carcinogens and allow a detailed map of "hot spots" within these sequences. In addition, the experience will provide approaches for isolating other genes from human DNA.

METHODS EMPLOYED: DNA isolation techniques including enzymatic digestion, phenol extraction, shearing, Hg^{2+} - and Ag^{+} Cs_2SO_4 density gradient centrifugation: Actinomycin D - CsCl density gradient centrifugation; DNA-RNA hybridization.

MAJOR FINDINGS: To date we have been able to prepare DNA from human placenta, shear it to defined size and separate the rDNA genes from the bulk of the DNA in Hg^{2+} - Cs_2SO_4 gradients (see project on Interaction of Heavy Metal Ions. .). This finding combined with selective thermal denaturation techniques developed in Stafford's laboratory has allowed the partial purification of these genes. Although our preparations are not yet pure, restriction nuclease (Eco RI) digestion patterns have revealed four fragments. Currently we are working up larger batches of material and characterizing these fragments.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Analysis of mutagenesis in human cells requires knowledge of the structure of human genes and their susceptibility to attack by mutagens and carcinogens. Any progress towards this goal would be of great importance in our understanding of the molecular events involved in mutation in man.

PROPOSED COURSE: To carry out the isolation of the rDNA sequences from human material and their properties; to investigate the interaction of these sequences with carcinogens and mutagens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60019-03 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The use of male germinal tissue in the detection of mutational events

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. V. Malling	Section Head	EMB NIEHS
OTHER:	N. Mitra	Visiting Fellow	EMB NIEHS
	J. S. H. Elkington	Visiting Fellow	EMB NIEHS

COOPERATING UNITS (if any)
Environmental Biology & Chemistry Branch
Biometry Branch

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to develop systems for measuring the frequency of point mutations and DNA loss in male germinal tissue. These systems would have the advantage of testing the human population by means of readily available samples, i.e., spermatozoa. At present enzyme histochemical methods are being developed to detect point mutations in male mice and three enzymes and their inhibitors have been characterized. These enzymes are α-glycerophosphate dehydrogenase, lactate dehydrogenase and succinate dehydrogenase. Preliminary work has started with the FACS-1 cell sorter to determine its usefulness in measuring the distribution of DNA content in a population of sperm. It is hoped that this instrument may prove useful in showing changes in the distribution pattern in sperm populations derived from mice treated or untreated with known mutagens.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The aim of this project is to develop systems for measuring the frequency of point mutations in male germinal tissue by sampling the sperm. These studies use enzyme histochemical techniques and would have the advantage of testing the human population by means of readily available samples, i.e., spermatozoa, and would provide an assay procedure for determining the effects of chronic levels of exposure to a suspected mutagen, as the genetic damage may accumulate in the stem cell spermatogonia.

METHODS EMPLOYED: Some enzymes in sperm can be readily detected by histochemical methods. The enzymes under study in this laboratory are α-glycerophosphate dehydrogenase, lactate dehydrogenase, succinate dehydrogenase, isocitrate dehydrogenase and sorbitol dehydrogenase.

The principle of these studies is that, with the use of standardized staining procedures and variations in coenzymes and by using specific enzyme inhibitors, it should be feasible to detect active site mutations in the spermatozoal enzymes. These mutations will be estimated by counting stained and unstained cells with the aid of a Zeiss axiomat microscope connected to a PDP-12 Computer and a teleprinter output. Information will also be collected on magnetic tape for processing in the institute's PDP-11 computer.

MAJOR FINDINGS: At present, it has been possible to standardize the staining technique to the extent that it is reproducible after repeated experimentation. Apart from the three enzymes described later, isocitrate dehydrogenase can now be reproducibly stained but no specific inhibitors have been tested. It has also been possible to develop a system of inhibitors specific for three of the enzymes being tested. They are listed below in accordance with the enzyme.

Enzyme	Inhibitor
1. α-glycerophosphate dehydrogenase	ATP Oxamic acid Malonic acid
2. Lactic dehydrogenase	Urea, Oxamic acid
3. Succinic dehydrogenase	Malonic acid

Experiments are being done with known mutagens to test the validity of the system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE: Development of this system is important, because it would permit the detection of mutational events within a single individual that could be used to monitor the human population. Besides, it would also enable us to detect the genetic consequences of exposure to mutagens.

PROPOSED COURSE: The future plans include testing out the present system to determine the spontaneous frequency of variant cells as well as the frequencies induced by various mutagenic agents. Other enzymes and their inhibitors will also be investigated and preliminary work will start on using the FACS-1 cell sorter to monitor the proportion of stained and unstained cells as well as the distribution of DNA content within populations of mouse sperm.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60020-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Identifying of Chemical Compounds Activated by Germ Cells to Mutagens in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter Maier	Visiting Fellow	EMB NIEHS
OTHER:	Heinrich Malling	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to identify compounds which induce point mutations, after metabolic activation by testicular tissues to mutagens in mammals. The topics are: (1) Screening of the literature for compounds which react with testicular tissue, (2) Development of protocols and determination of optimal conditions for the in vitro mutation experiments, (3) Characterization of the activation capacity of germinal tissue in vitro and (4) Correlation with a histochemical mutation system using sperm of male mice. As a result we found that AF-2, a nitrofuran, is activated in the soluble enzyme system and the microsomal fraction of rabbit testis-tissue.

PROJECT DESCRIPTION

OBJECTIVES: Initially this research will attempt to identify compounds which induce point mutations after metabolic activation in the testis of male mice and to develop the protocols necessary for this work. The first series of compounds to be tested will be those which are known to react with testicular tissue. Any compounds that give a high level of mutagenic activation in the testicular microsome test will be tested in a histochemical mutation system using sperm of male mice.

METHODS EMPLOYED: The systems employed are a homogenate, a soluble enzyme system and a pure microsomal fraction of testis from rabbits. Bacteria and phages were used as indicator organisms for mutations. Also to be used will be a histochemical mutation system in which individual sperm cells are examined for a mutational event.

MAJOR FINDINGS: According to screening of the literature, nitrofurans interact with testicular tissue in mammals. Due to several pilot experiments, the food additive, AF-2, was chosen as a test compound and the multipurpose strain E. coli 343/113 as an indicator. As a metabolic activation system we used a 20,000 g and a 176,000 g supernatant and washed microsomes from New Zealand rabbit testis. With and without homogenate we found different mutation patterns. The mutation rate in one locus of the microorganisms is 5x higher with the soluble enzyme system and 2x higher with the microsomal fraction than without. According to the modifying effect of incubation atmosphere, cofactors and inhibitors, we conclude that there is, among others, a nitroreductase involved.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is of importance to future generations to identify compounds that cause point mutations in germinal tissue. A compound which is highly activated by testicular tissue homogenates and induces mutations in indicator organisms is likely to induce point mutations in sperm. Such systems as these will aid in preventing unnecessary future genetic hazards.

PROPOSED COURSE: In order to separate and eliminate the interaction between the microorganism metabolism and mammal metabolism the following investigations are planned: (1) extracellular induction of mutations in phages, (2) tests with the histochemical mutation system. Both systems are still under development in other laboratories.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60021-04 EMB

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Electrophoretic Mobility Mutation Analysis in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lawrence R. Valcovic	Actg. Assist. Chief	EMB NIEHS
OTHER:	Heinrich V. Malling	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	1.1	2.1

SUMMARY OF WORK (200 words or less - underline keywords)

The study of specific enzyme mutations using electrophoresis has the capability of detecting mutations that result in either a non-functional protein or a functional protein with altered electrical charge. Our objective is to develop a single mammalian test system that will detect both electrophoretic mobility (EPM) and inactive enzyme (IAE) mutations in the same loci. Two inbred mouse strains which differ with respect to nine enzyme loci have been selected for this system. Adult males of each strain were treated with gamma radiation and mated to unirradiated females of the other strain to produce F_1 hybrid offspring. Tissue samples are obtained surgically and electrophoresis is conducted on crude homogenates. While sampling is not yet completed, preliminary data indicate that the system is at least as sensitive in detecting IAE mutations as is the morphological specific locus system. Several mutants have been obtained and currently studies are underway to determine the histochemical nature of the induced mutations.

PROJECT DESCRIPTION

OBJECTIVES: Classically, the detection of point mutations in mammals has relied upon the induction of visible recessive mutations. These studies have not yet identified the molecular event responsible for producing the mutant phenotype. However, the study of specific enzyme mutations using electrophoresis has the capability of detecting mutations that result in a non-functional protein or a function protein with altered electrical charge. Our objective is to develop a single mammalian test system that will detect both electrophoretic mobility (EPM) and inactive enzyme (IAE) mutations in the same loci. This will enable us to determine the relative frequencies of these two types of mutational events in response to various types of mutagenic agents.

METHODS EMPLOYED: The ratio between mutations resulting in IAE-mutations and EPM-mutations can be estimated from the changes in the zymogram of heterozygous offspring from a cross between two inbred strains which differ in the electrophoretic patterns of several enzymes. In the simplest case let us assume the normal heterozygote will have three bands for a particular enzyme. A mutant induced in either parent can result in one of three different electrophoretic patterns: (a) the presence of any one band indicating that the mutant is inactive; (b) the presence of any two bands, one at the normal position for one of the parents and the other at any position indicating that the inactive enzyme will form an active aggregate with the wild-type enzyme; and (3) the presence of three bands, two with changed positions, indicating an EPM-mutation. Since case (a) and (b) indicate inactive enzymes and (c) an EPM-enzyme, a direct estimation of the ratio between IAE-mutations and EPM-mutations can be made.

We have selected two inbred mouse strains which differ with respect to nine enzyme loci. Additional non-variant bands will be apparent with some of the strains; and while the number of loci that these represent is unknown, EPM-mutations may be detected at these positions.

Since it is important to correlate this system with Russell's specific locus test in mice, the first mutagen treatment utilized was ionizing radiation. Adult males were irradiated and after a sterile period ranging from 4 to 11 months they were mated to non-irradiated females. The animals received a total of 1000r ^{60}Co gamma rays delivered as two 500r exposures with a dose rate of 90r/min. separated by 24 hours.

Sampling of progeny consists of obtaining blood by orbital puncture and one kidney by surgery. Subsequently, electrophoresis is carried out on the red cell lysate and kidney homogenate from each animal. It is anticipated that approximately 3000 offspring from each of the crosses (DBA x C57; C57 x DBA) will be analyzed for mutations at each of the nine loci showing electrophoretic polymorphism.

MAJOR FINDINGS: Testing for mutations in offspring from DBA/2J treated males mated to C57BL/6J untreated females has progressed. At this writing approximately 2600 animals (24,400 loci) have been examined. Four apparent mutations at different loci have been detected. This tentative mutation rate of .8 x 10^{-4} mutations per locus per gamete is not dissimilar to that obtained in the morphological specific locus test.

Additional treatment of C57BL/6 males at lower radiation doses was completed. Exposure to 700r total dose (350r + 350r) also resulted in extremely high sterility. Further radiation will not be done due to the amount of data being generated from the DBA/2J treated group.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The widespread use of chemicals may increase the genetic load of the human population which may possibly present a health hazard. There are two approaches that may be considered to avoid such an event. First, use animal test systems to detect chemicals which are mutagenic and prohibit or limit their use. Second, use mutant alleles at selected loci in the human population and monitor these for several years to determine whether there is an increase in the frequency of these alleles. The system under development will be pertinent to both of these approaches.

It will survey a large number of different loci to minimize the cost of testing chemicals for potential mutagenicity; and since many of the enzymes are also found in the human population, it will also provide a base-line for the study of the mutation rate of electrophoretic mobility mutations in the human population. Furthermore, since most of the loci code for specific enzymes, purification and biochemical analyses can be employed to determine the nature of the mutational event, i.e., base-pair substitution and/or addition/deletion in the DNA.

PROPOSED COURSE: The radiation experiment will be completed in FY76. Breeding of mutants to obtain homozygotes for biochemical analysis will continue. Procedures for exposing animals to chemical mutagens are being developed and treatment of males of both strains is planned. Efforts to increase the sensitivity of the system by increasing the number of test loci will continue.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60022-02 EMB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Heat Stability of Enzymes: An Approach to Measuring Point Mutations in the Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jean-Claude Mareschal	Visiting Fellow	EMB NIEHS
OTHER:	Lawrence R. Valcovic	Actg. Assist. Chief	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Mutagenesis Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The heat-stability properties of enzymes are being used to develop a point mutation system for use in mammalian organisms. The enzymes are examined spectrophotometrically and heat-stability curves are plotted. Several important differences between enzymes from different inbred strains of mice have been found. The findings of this study will be expanded and will then be used to search for mutant offspring of chemically treated parents.

PROJECT DESCRIPTION

OBJECTIVES: Point mutations within inbred strains of mice will be studied using some biochemical properties and/or the heat stability properties of enzymes. These studies will provide an increased number of loci to be examined in mouse biochemical specific locus tests.

METHODS EMPLOYED: After incubating the mouse kidney homogenate of different mouse strains at given temperatures for a specific time period, the enzymes were assayed spectrophotometrically. Curves were plotted for each set of conditions and each enzyme to determine any heat stability differences. Samples were also examined using electrophoresis.

MAJOR FINDINGS: Twenty enzymes have been studied in this project and one half of them show significant heat stability differences between the two mouse strains. On the basis of these studies phosphoglycerate kinase and glucose-6-phosphate have been shown to be X-linked in the mouse.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Heat-stability studies will expand the number of loci that can be studied in the mouse biochemical specific locus mutation system. This will allow the assay to be more sensitive, less expensive and less time consuming. These studies will also aid mapping of new loci in the mouse genome.

PROPOSED COURSE: Analysis of F_1 animals will be pursued to determine the heat stability patterns of the heterozygote. Biochemical studies will be initiated in an effort to determine the molecular basis for the observed heat stability differences.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60023-02 EMB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Comparison and Evaluation of Mutagenic Test Systems for Mass Screening Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Larry Claxton	Biologist	EMB NIEHS
Other:	H. V. Malling	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Mutagenesis Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.1	0.7

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to compare the usefulness and scientific reliability of several screening systems for mutagenicity. The work will compare a variety of bacterial systems, activation systems and host-mediated assays. The microsomal activation system's format and data analysis are in the process of being computerized in order to facilitate objective analysis.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this study is to evaluate and compare the usefulness, reliability and scientific soundness of presently available mutation test systems using a variety of mutagenic agents. This project will attempt to provide standardized protocols and computerized control of tests that can be used in future hierarchal test systems commonly called Tier tests. This evaluation would aid in the comparison of results from several test systems and in the extrapolation to human studies. The computer programming of test systems would insure objective analysis and comparison of data which is not presently available.

METHODS EMPLOYED: Several known mutagens that have different mechanisms of genetic action and different physical and chemical characteristics are being tested in-house in each of the selected test systems. The parameters of each system are varied in such a manner as to standardize the system for screening conditions. A comparison is then made between the sensitivity, reliability, etc., of the various systems used. Included within the systems being tested are several bacterial systems, activation systems and a host-mediated assay system.

MAJOR FINDINGS: The microsomal activation system has been investigated and a standardized protocol for general testing has been produced. The computerization of the microsomal test system is in its final stages and should be completed before June 30, 1976. Initial work on the host-mediated assays has begun.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This evaluation and comparison will provide scientifically sound basis for large-scale screening and testing of environmental chemicals for mutagenicity. This study will also provide answers as to the feasibility of extrapolating results from one system to another.

PROPOSED COURSE: Future efforts of this project will be to standardize other test systems now being evaluated and determining the proper controls for mass screening. The computerized microsomal system will also be evaluated.

PUBLICATIONS

Claxton, L. D. and Barry, P.: Public Health Perspectives of Environmental Mutagens. American Journal Public Health (in preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60024-02 EMB

PERIOD COVERED

July 1, 1975 to December 31, 1975

TITLE OF PROJECT (80 characters or less)

Effects of dietary deficiency on sensitivity to mutation induction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Section Head	EMB NIEHS
OTHER:	E. R. Soares	Senior Staff Fellow	EMB NIEHS
	R. M. Sorg	Geneticist	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Mutagenesis Branch

SECTION

Mammalian Genetics & Cytogenetics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study was to investigate whether dietary deficiency would lead to a change in the susceptibility to mutagenic treatment. Mice raised on a Vitamin D deficient diet and their counterpart raised on a fully supplemented diet were treated with a known chemical mutagen. Induction of dominant lethals and chromosome aberrations were compared.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: In irradiation studies with mice, it has been found that diets deficient in one or more of the vitamins or trace minerals lead to an increase of the somatic sensitivity of the animal to induction of damage. Somatic and genetic sensitivity have been shown to be correlated in the mouse It is our purpose to investigate whether or not dietary deficiency might change the overall state of the animal making it more susceptible to the actions of mutagenic agents.

METHODS EMPLOYED: Inbred mice of the C57BL/6 strain were placed on a diet deficient in Vitamin D and after an appropriate adaptation time were mated in single pair matings for 100 days. F_1 offspring were continued on the diet until maturity. Male offspring were treated with a known chemical mutagen (TEM) and outcrossed in a dominant lethal test. Male offspring were also treated with the agent and bred to CD_1 females to produce offspring for cytogenetic analysis of the induction of transmissible translocations.

MAJOR FINDINGS: No significant increases in the frequency of induced dominant lethals or the frequency of transmissible translocations were detected among offspring to animals raised on the deficient diet compared with animals raised on the full diet following mutagenic treatment of the parents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A greater part of the human population on a world-wide basis is known to be subjected to nutritional insufficiency at the present time. At the same time, it is known that there are present in the human environment compounds which are mutagenic. Knowledge of the existence of effects of dietary deficiency on sensitivity to mutation induction will be a necessary consideration in attempts to extrapolate from information derived from laboratory animals in estimating risks to the human population.

PROPOSED COURSE: Further studies encompassing other deficiencies will be deferred until similar ongoing collaborative studies in Drosophila melanogaster have been completed and are evaluated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60026-03 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Comparative sensitivities of different mouse strains to mutagenic agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. R. Soares	Senior Staff Fellow	EMB NIEHS
	W. Sheridan	Section Head	EMB NIEHS
OTHER:	None		

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Mammalian Genetics and Cytogenetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier studies, utilizing irradiation as the mutagenic source have shown that the genome of the test organism is a major factor in the determination of its susceptability to genetic damage. Preliminary screening of five inbred strains in our laboratory, using chemical mutagens indicate the existence of strain differences between these strains of mice. Future efforts will include retests of these strains plus additional strains. In addition, efforts will be devoted to understanding the underlying genetic causes of the observed differences.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: Earlier studies, utilizing irradiation as the mutagenic source, have shown that the genome of the test organism is a major factor in the determination of its susceptibility to genetic damage. It is our purpose to investigate to what extent such genetically caused differences in sensitivity pertain to other mutagenic agents. We also want to know if the relative level of sensitivity of a defined genome to induction of mutations is constant for all agents or variable depending on the mutagen used.

METHODS EMPLOYED: We want to accrue a battery of test strains, each showing a distinct level of sensitivity to mutation-induction, covering a range from highly sensitive to highly resistant. These strains will be selected on the basis of induction of dominant lethal mutations and increases in the percent of morphologically aberrant sperm by known potent mutagens. They will then be challenged by a successive array of other known or suspected mutagenic agents to discover any changes in their relative levels of sensitivity or indications that suspect compounds may be mutagenic.

MAJOR FINDINGS: Preliminary screening of five inbred strains has been made. There are indications of differences between some of the strains tested. These differences give promise of the success of the project; however, retests of these strains plus additional strains will be necessary before final choices of strains to be included in the test battery are made.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since the gene pools of human populations by and large are extremely heterogeneous, one would expect a broad spectrum of reactions to a mutagenic agent in the environment. Restriction of testing to single, often inbred, strains of another mammal such as the mouse will not give indications on a wide scale of what might be the effects of such an agent on the human population. By determining the effects of a compound on a battery of strains presenting a variety of genotypes and differing sensitivities, we will better be able to approximate what might be expected in a heterogeneous mammalian population such as man.

PROPOSED COURSE: Future efforts will be devoted to understanding the underlying genetic causes of differences in sensitivity to mutation induction, and the consequences of combining the genetic materials of strains with divergent sensitivities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60027-03 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Comparisons of effects of chemical mutagens on somatic and germ cells of mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Section Head	EMB NIEHS
	E. R. Soares	Senior Staff Fellow	EMB NIEHS
OTHER:	R. M. Sorg	Geneticist	EMB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Mammalian Genetics & Cytogenetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to explore the feasibility of using somatic cells to screen for chemically induced chromosomal aberrations. Included in this project are comparisons with respect to the efficiency of detection of chromosome aberrations in germ cells (testicular preparations and sperm morphology), cultured lymphocytes and bone marrow preparations.

PROJECT DESCRIPTION

OBJECTIVES: This project is designed to explore the feasibility of using somatic cells to screen for chemically induced chromosome abnormalities. The use of somatic cells for screening purposes would allow for rapid screening of large numbers of animals provided such techniques are reliable in the detection of aberrations. Included in this project are comparisons with respect to the efficiency of detection of chromosomal aberrations in germ cells and cultured lymphocytes and/or bone marrow.

METHODS EMPLOYED: Lymphocytes cultured from offspring of treated males are prepared for cytological examination and karyotyping. Similarly, testicular sections and air dried preparations of testicular tissues as well as sperm from the vas and epididymis are examined for chromosome abnormalities and sperm head abnormalities respectively.

MAJOR FINDINGS: In the studies conducted thus far, chromosome preparations from cultured lymphocytes or bone marrow seem to be less reliable for use in the detection of aberrations than chromosomes prepared from testicular tissues. Lymphocyte preparations are most useful in those cases where the chromosomal aberration (translocation) results in the production of (1) small fragments, (2) metacentric chromosomes or (3) chromosomes with disproportionately large or small arm lengths relative to the control karyotypes. In functionally sterile males suspected to be translocation carriers, we found upon examination of sperm that (1) actual counts of sperm were severely reduced and/or (2) as many as 90% of the sperm present were abnormally shaped.

SIGNIFICANGE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although chromosome abnormalities are known to exist and be transmitted to following generations, very little is known of the long-term effects of such aberrations. Studies of the effects of heritable chromosome aberrations in mice will foster a better understanding of the mode of action and significance to the well being of the animal of such abnormalities.

PROPOSED COURSE: The mutagenic effects in mice of chemical agents will be tested. The mode of action and the long-term effects of induced, heritable chromosomal abnormalities will be studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60028-02 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Genetic Effects on Early Mouse Embryos after Mutagenic Treatment of the Parents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Burki	Visiting Fellow	EMB NIEHS
	W. Sheridan	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Mammalian Genetics & Cytogenetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the underlying causes of induced embryonic mortality in the dominant lethal test in the mouse. This is achieved by direct observation of the in vitro development of embryos and by cytogenetic analysis of their karyotypes.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: One of the most widely used tests of mutagenicity in mammals is the dominant lethal assay. While this system allows detection of induced increases in foetal mortality, the underlying causes are not clearly delineated. One of the proposed factors has been the induction of chromosomal aberrations. It is our purpose to investigate the processes and causes of foetal mortality by direct observation of the development of embryos and by cytogenetic analysis of their karyotypes.

METHODS EMPLOYED: The methodologies of culturing mouse embryos in vitro up to the blastocyst stage and on to the outgrowth of the trophoblast are used. Male mice are treated with a mutagen (TEM) and mated to untreated females to allow in vivo fertilization. Approximately thirty-two hours post mating the two-cell embryos are retrieved from the oviducts of the females and established in cultures. An air-drying method is used to make cytological preparations from in vivo and in vitro grown embryos at various stages of development.

MAJOR FINDINGS: Treatment of the males did not reduce the rate of fertilized and cleaving eggs but affected further development of the embryos at all stages. The percentages of cultured embryos showing normal development decreased with increasing dose of TEM injected and varied with the time of conception post-injection for treated post meiotic stages of spermatogenesis. The evaluation of the cytological preparations is under way.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chromosome aberrations in man are known to cause disturbances in development of embryos leading to death of the embryo or to abnormal offspring. The project described here is designed to develop an understanding of the fate and effects of spontaneous and induced chromosome aberrations at various stages of embryogenesis. The system when fully developed may permit the detection of possible mutagenic effects of potential mutagens in the human environment.

PROPOSED COURSE: After treatment of germ cells the specific pattern of morphological and cytological aberrations of early mouse embryos induced by different known chemical mutagens will be compared. The in vitro culturing of embryos will also be used to observe directly the development of embryos carrying a known inherited mutation.

PUBLICATIONS

Burki, K., Sheridan, W. and Soares, E.R.: In Vitro Embryo Culture and Chemical Mutagenesis. Mouse News Letter, February 1976 (abstract).

Burki, K. and Sheridan, W.: In Vitro Culture of Mouse Embryos After TEM-Treatment of the Fathers. Seventh Annual Meeting of the Environmental Mutagen Society, March 1976 (abstract).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60029-03 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Genetic Variability and Its Relationship to Environmental Variation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John H. Gillespie	Senior Staff Fellow	EMB NIEHS
	Charles H. Langley	Senior Staff Fellow	EMB NIEHS
OTHER:	Harry Guess	Senior Staff Fellow	EBB NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Population Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

SUMMARY OF WORK (200 words or less - underline keywords)

One important consideration in evaluating the significance of genetic variation is environmental variation in selection. This work is aimed at the theoretical modelling of population of various structure and under various regimes of natural selection. Such theoretical considerations point the way for experimental design and confirmation.

PROJECT DESCRIPTION

OBJECTIVES: A recognized deficiency in classical population genetics is the constancy of selection parameters. The purpose of the project is to relax this assumption in a manner which is meaningful biologically. Many assayable enzymes show electrophoretic polymorphism. The work in this project incorporates enzymological-physiological considerations into the effect of environmental variation on the fitness effects of enzyme variation.

METHODS EMPLOYED: This work depends primarily on the application of basic stochastic process theory to population genetics models.

MAJOR FINDINGS: It has been possible to incorporate autocorrelated environmental variation into model through the development of some novel approaches. Consideration of multiple alleles and the relationship between enzyme activity and physiological effect has produced new possible outcomes under these models.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In order to evaluate the expected consequences of increases in the mutation rate, it is important that we have a plausible theory with which the diverse observations of population genetic variation can be organized. There are still many gaps in our knowledge of the extent and biological significance of population genetic variation. For the present, theoretical work such as this provides some guide in public health considerations and in further biomedical research in the areas of medical genetics and environmental research.

PROPOSED COURSE: Since the principle investigator has left NIH, this project will proceed only as a small collaborative effort.

PUBLICATIONS

Gillespie, J. (1975) Natural selection for within-generation variance in offspring number. II. Discrete haploid models. Genetics 81:403-413.

Gillespie, J.H. and C.H. Langley: Multilocus behavior in random environments. I. Random Levene Models (accepted in Genetics).

Gillespie, J.H. (1975) A general model to account for enzyme variation in natural populations. II. Characterization of the fitness function. American Naturalist (in press).

Crump, K.S. and J.H. Gillespie (1975) The dispersion of a neutral allele considered as a branching process. J. Applied Probl. (in press).

Gillespie, J.H. (1976) A general model to account for enzyme variation in natural populations. III. Multiple alleles. Evolution (submitted).

Gillespie, J.H. (1976) The role of migration in the genetic structure of populations in temporally and spatially fluctuating environments. II. Island models. Theor. Pop. Biol. (submitted).

Gillespie, J.H. and H.A. Guess (1976) The effects of autocorrelation on the progress of selection in temporally fluctuating environments. (Submitted).

Crump, K.S. and J.H. Gillespie (1975) Spatial autocorrelations for neutral alleles. Theor. Pop. Biol. (submitted).

Guess, H.A. and J. Gillespie (1976) Diffusion approximations to solutions of stochastid differences equations with autocorrelated environments. J. Applied Prob. (submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60033-01 EMB

PERIOD COVERED
January 1, 1976 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Simultaneous Detection of a Variety of Genetic Events in Aspergillus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Barry R. Scott Visiting Fellow EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

At present there is no system which can simultaneously detect both forward mutation and chromosome aberrations. This program is designed to fill this lacuna, such that at least 50 plus genetic events can be detected in one organism, Aspergillus nidulans - medium system.

PROJECT DESCRIPTION

OBJECTIVES: These are to construct a diploid strain of Aspergillus heterozygous for various loci and to elucidate the types of induced mutagenic events induced in this strain by various standard mutagens.

METHODS EMPLOYED: Routine genetic manipulations will be employed. Associated with this technique used in handling Aspergillus nidulans for obtaining quantitative mutation and survival data (see Scott, Alderson and Papworth: Radiation Botany (1972) 12:45-50; J. Gen. Microbiol. (1973) 75:235-239; Scott and Alderson: J. Gen. Microbiol. (1974) 85:173-176).

MAJOR FINDINGS: No information available because project has just been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Overall at least 50 plus forward mutational events should be detected by the above system. These events range from point mutation to chromosome aberrations and other genetic events by a single initial detection method. At the most simplistic level such a system could be a very powerful tool in detecting deleterious agents inducing genetic changes. While at the most advanced level it should be possible to determine whether or not there is a correlation between the various types of possible genetic alterations ranging from point mutations to viable chromosome aberrations and other genetic events which are important to mankind.

PROPOSED COURSE: (1) The diploid heterozygous strain will be constructed. (2) Once established the types of genetic events induced by various mutagens will be studied. (3) To expand the data banks with various known and unknown chemical mutagens/carcinogens, especially those of high relevance to man and (4) To use this system in various host-mediated and microsomal activation systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60034-01 EMB

PERIOD COVERED
January 1, 1976 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Detection of Air Pollutants and Low Levels of Mutagens/Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Barry R. Scott	Visiting Fellow	EMB NIEHS
OTHER:	Arnold Sparrow		Brookhaven Nat'l. Lab.
	Susan Lamm		Brookhaven Nat'l. Lab.
	Lloyd Schairer		Brookhaven Nat'l. Lab.
	Paul Klotz		Brookhaven Nat'l. Lab.

COOPERATING UNITS (if any)
Brookhaven National Laboratory, Upton, NY

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

"Low, repeated doses of such common place pollutants as fumes in the air and chemicals in drinking water are increasing risks of cancer, heart disease and genetic mutations," according to a library of congress report. This report also indicates that the existing testing methods are expensive, arduous and not sensitive enough to detect polluting agents. The primary aim of this project is to attempt to develop inexpensive biomonitors to detect these hazardous environmental agents and compare the results obtained with these systems with the work already in progress at Brookhaven using Tradescantia.

PROJECT DESCRIPTION

OBJECTIVES: To establish test system(s) and test methods with microorganisms which are capable of detecting air pollutants and low levels of mutagens/carcinogens. Once established these system(s) and methods will be tested in the field.

METHODS EMPLOYED: Established screening systems which detect mutagens are being employed in the first part of the investigation. These systems employ the organisms, Salmonella typhimurium, Escherichia coli, Neurospora crassa and Aspergillus nidulans.

MAJOR FINDINGS: No information available because project has just been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The evolution of inexpensive biomonitor to detect hazardous environmental agents which induce genetic damage after chronic exposure to mutagens is urgently required. Such monitors once evolved could be routinely used to screen for air pollutants, act as early warning systems and possibly be used to identify the deleterious agents involved.

PROPOSED COURSE: A series of steps are planned, these include: (1) an initial investigation of a series of established microbial test systems to determine the limitations of each in detecting the mutagenic activity of 1,2-dibromoethane (a known fumigant and petrol additive) in the ppm range under laboratory conditions, (2) determining the parameters which affect the detection of air pollutants and chronic exposures of mutagens in the most promising test system which might be used as biomonitors, (3) expansion of the data bank with selected systems treated with other mutagens and carcinogens and (4) an in-the-field trial of the systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60035-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Development of Test Systems in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David F. Callen Visiting Fellow EMB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Ongoing experiments to modify or generate new test systems in yeast include (1) including the UV sensitive gene rad18 in the strain D4, (2) growing yeast anaerobically to alter permeability and (3) identification of two linked recessive resistance genes to contrauct a strain to monitor non-disjunction.

PHS-6040

PROJECT DESCRIPTION

OBJECTIVES: It should be possible to increase the sensitivity of the yeast strain D4 by, (1) including the UV sensitive gene rad_{18} in the strain. From the results of other workers it is known that a strain possessing this gene will be more sensitive than wild-type to a variety of mutagens. (2) Increasing the permeability of the strain D4 to mutagens. When the yeast cell is grown anaerobically the lipids become depleted. Since lipids are components of the outer membrane the permeability of this membrane may change. Although many systems are available for monitoring mutation or gene conversion, there is no simple method for detecting changes involving whole chromosomes. It is proposed to develop a method for monitoring non-disjunction in yeast by constructing a diploid strain heterozygous for two linked recessive resistance genes.

METHODS EMPLOYED: Haploids were isolated from diploids by dissecting with a micromanipulator. Genotypes of haploid and diploid strains were tested by the standard technique of replicating to omission media. The segregation of the rad_{18} gene could be detected by comparing the growth of cell droplets spread on plates which had been irradiated with UV light. The yeast cells were treated in liquid suspension by shaking at 30°C.

MAJOR FINDINGS: (1) Crosses to include the radiation sensitive gene rad_{18} in the strain D4 have now been completed. (2) Experiments have indicated that anaerobically grown cells are 3 to 4 times more sensitive to the induction of gene convertants by mitomycin C and ICR-170 than are aerobic cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Short-term mutagenicity tests with a particular environmental agent are more reliable when data has been derived from a variety of organisms. Yeast has the added advantage over bacterial systems that it is a eukaryote and, therefore, the cellular and genetic responses to mutagenic agents may more closely resemble those of higher cells.

The yeast strain D4 is used to monitor gene conversion induced by mutagenic agents but this system is relatively insensitive when compared to test systems in some other organisms. Thus, yeast would play a more important role in short-term testing if the sensitivity of this strain could be increased.

PROPOSED COURSE: (1) The sensitivity to various mutagens of the strain D4 and the related strain which is homozygous for the rad_{18} gene, will be compared to ascertain if the radiation sensitive gene has resulted in any significant increase in sensitivity. (2) The increased sensitivity to mutagens of anaerobically grown cells as compared with aerobically grown cells will be further tested by examining the effect of additional mutagens. To monitor non-disjunction two linked recessive resistance genes are necessary. Work is in progress to identify two such genes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60036-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Genetic Activity of Environmental Agents in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David F. Callen	Visiting Fellow	EMB NIEHS
OTHER:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS
	Georges R. Mohn	Visiting Scientist	EMB NIEHS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Studies with yeast indicated that (1) a drug, F30066, used in China for the treatment of schistosomiasis, was genetically active and (2) of three structurally related nitroimidazoles tested, one was found to be genetically active in the yeast system.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To investigate the genetic activity of the following environmental agents in the yeast, Saccharomyces cerevisiae, (1) three structurally related nitroimidazoles which have been synthesized as a possible replacement of the drug metronidazole (Flagyl), (2) F30066 an antischistosomiasis agent used to treat people in China.

METHODS EMPLOYED: The yeast strain D4 was used to monitor gene conversion at the trp-5 and ade-2 loci. Yeast cells and various concentrations of the test agent were incubated together in liquid suspension. After a given time the cells were harvested and plated on appropriate media.

MAJOR FINDINGS: (1) Of the three nitroimidazoles tested (25095, 26306 and 26173) only 26173 was genetically active in the yeast system. This compound, at a concentration of 13·3 µg/ml gave approximately a 3-fold increase over the spontaneous conversion frequency at a cell survival of 75%. (2) F30066 was genetically active in yeast. For example, in one experiment F30066 at a concentration of 13·3 µg/ml was found to result in a 6-fold increase over the spontaneous conversion frequency at a cell survival of 30%.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: (1) Of the three nitroimidazoles tested only one was found to be genetically active in the yeast system. These results are part of a preliminary screen for the genetic activity of these compounds before their evaluation as possible drugs for use in man. (2) The antischistosomiasis agent F30066 is used in China. These results suggest that this compound may be a genetic hazard. Continued use of this drug in treating the human population would be inadvisable without further evaluation of its genetic and carcinogenic hazard.

PROPOSED COURSE: This project has now been completed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60037-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Genetic Activity of Aflatoxins in Yeast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David F. Callen	Visiting Fellow	EMB NIEHS
OTHER:	Tong-man Ong	Senior Staff Fellow	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the genetic activity of aflatoxins and their metabolites in the yeast Saccharomyces cerevisiae. The topics of present interest are: (1) Comparison of the genetic activity of aflatoxin B_1 and G_1 when metabolized by hamster liver homogenate; (2) An investigation of the metabolism of aflatoxin B_1 by an alteration of epoxide hydrase activity with the inhibitor 1,2-epoxy-3,3,3-trichloropropane, and (3) Activation of aflatoxin B_1 by yeast cells and a possible correlation with the cytochrome P-450 content of yeast.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: Aflatoxins are highly carcinogenic substances and can occur as contaminants in food. (1) Since aflatoxin B_1 has been shown to be a more potent carcinogen than aflatoxin G_1, it is of interest to compare their genetic activity in yeast. (2) The active metabolite of aflatoxin B_1 is thought to be an epoxide which can be converted to a dihydrodial by epoxide hydrase, an enzyme present in liver homogenate. 1,2-epoxy-3,3,3-trichloropropane (ETP) is an inhibitor of the epoxide hydrase and therefore addition of this inhibitor to the mixture of aflatoxin B_1 and liver homogenate should increase the proportion of available epoxide which can react with indicator cells. (3) Yeast cells have been shown to possess cytochrome P-450 and the proportion of this cytochrome varies depending on the growth conditions of the cells. Current experiments have shown that aflatoxin B_1 is genetically active in growing yeast cells. It would be of interest to determine if the genetic activity of aflatoxin B_1, in the absence of an *in vitro* activating system, is correlated to the cellular cytochrome P-450 content.

METHODS EMPLOYED: The strain D4 of yeast was used. This diploid strain can be used to monitor gene conversion at the *ade-2* and *try-7* loci. In all experiments a liquid test system was used in which the yeast cells were incubated with varying concentrations of aflatoxin. Liver homogenate was prepared from phenobarbitol treated hamster.

MAJOR FINDINGS: (1) Aflatoxin B_1 at a concentration of 100 µg/ml increased the *ade* convertant frequency from $3{\cdot}3/10^6$ survivors to $93{\cdot}0/10^6$ survivors. In contrast aflatoxin G_1, even at 200 µg/ml, did not significantly increase the conversion frequency. Therefore, aflatoxin B_1 has a greater genetic activity than aflatoxin G_1. (2) The inhibitor ETP was found to be genetically active *per se*. However, in the presence of liver homogenate this activity could be eliminated, presumably due to binding of ETP to proteins within the homogenate. When inhibitor was added to liver homogenate and aflatoxin B_1 there was an increase in the conversion frequency with increasing concentrations of inhibitor. This supports the hypothesis that the active metabolite of aflatoxin B_1 is an epoxide. (3) Experiments are still in progress to correlate the cellular content of cytochrome P-450 and the genetic activity of aflatoxin B_1 in yeast cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The results provide additional evidence to support the observed correlation between mutagenicity and carcinogenicity. The effect of the inhibitor, ETP, supports the idea that an epoxide is the active metabolite of aflatoxin B_1. In these experiments the unstable epoxide is assayed by its genetic activity on the indicator yeast cells. Systems in which inhibitors or promotors can alter the availability of such unstable metabolic intermediates can, therefore, be assayed by changes in the response of indicator cells.

PROPOSED COURSE: Experiments are in progress to attempt to correlate cellular content of cytochrome P-450 and the activation of aflatoxin B_1 by yeast cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60038-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
In Vitro Activation and Aflatoxin Mutagenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Tong-man Ong Senior Staff Fellow EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Microbial Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Aflatoxin B_1 is known to be one of the most potent chemical carcinogens ever found in the human environment. This and other aflatoxins, unfortunately, are common contaminants in agricultural products. Studies on the carcinogenicity of aflatoxins in rats have shown that aflatoxin B_1 is more tumorgenic than aflatoxin G_1 in liver. The latter compound, however, induces more tumor formation than the former compound in male kidney. Neither aflatoxin B_1 nor G_1 are carcinogenic in the kidney of female rats. It would be of interest, therefore, to see (1) are more mutagenic metabolites being converted from aflatoxin B_1 than aflatoxin G_1 by rats liver homogenates? (2) are more mutagenic metabolites being converted from aflatoxin G_1 than aflatoxin B_1 by kidney homogenates of male rats? (3) are kidney homogenates from female rats failing to convert aflatoxins B_1 and G_1 to mutagenic metabolites? (4) does epoxy hydrase affect the presence of mutagenic metabolites? and (5) is 2,3 epoxide an active form of aflatoxin metabolites?

Conidia are treated with aflatoxin B_1 or G_1, rat liver or kidney homogenates and a NADPH generating system at 37°C for 2 hours. Epoxy hydrase or epoxy hydrase inhibitor might also be added to the treatment solution. At the end of the treated conidia are washed. Treated and untreated conidia are assayed for the presence of ad-3 mutants by the direct method.

PROJECT DESCRIPTION

OBJECTIVES: Aflatoxin B_1 is known to be one of the most potent chemical carcinogens ever found in the human environment. This and other aflatoxins, unfortunately, are common contaminants in agricultural products. Studies on the carcinogenicity of aflatoxins in rats have shown that aflatoxin B_1 is more tumorgenic than aflatoxin G_1 in liver. The latter compound, however, induces more tumor formation than the former compound in male kidney. Both aflatoxins B_1 and G_1 are not carcinogenic in the kidney of female rats. It would be of interest, therefore, to see (1) are more mutagenic metabolites being converted from aflatoxin B_1 than aflatoxin G_1 by rats liver homogenates? (2) are more mutagenic metabolites being converted from aflatoxin G_1 than aflatoxin B_1 by kidney homogenates of male rats? (3) are kidney homogenates from female rats failing to convert aflatoxins B_1 and G_1 to mutagenic metabolites? (4) does epoxy hydrase affect the presence of mutagenic metabolites? and (5) is 2,3 epoxide an active form of aflatoxin metabolites?

METHODS EMPLOYED: Conidia from a genetically marked two-component heterokaryon of N. crassa will be harvested and treated with aflatoxin B_1 or G_1, rat liver or kidney homogenates and a NADPH generating system at 37°C for 2 hours. Epoxy hydrase or epoxy hydrase inhibitor might also be added to the treatment solution. At the end of the treatment, conidia will be washed. Treated and untreated conidia will be assayed for the presence of ad-3 mutants by the direct method.

MAJOR FINDINGS: Aflatoxins B_1 and G_1 can be converted by hamster or mouse liver homogenates to metabolites which are mutagenic in Neurospora. Addition of the epoxy hydrase inhibitor, 1,1,1,-trichloropropane 2,3 oxide (TCPO) to the test system increases ad-3 mutation frequency. These results seem to support the hypothesis that epoxide is an active form of aflatoxin. TCPO is a potent mutagen in Neurospora. The mutagenic activity of this epoxy hydrase inhibitor, however, diminishes if TCPO is incubated with liver homogenates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Aflatoxins are common contaminants in agricultural products. These compounds, therefore, are very closely related to human health. Studies described in this project will provide information in regard to the potential mutagenic hazard of these mycotoxins to man, in regard to the mechanism of aflatoxin mutagenesis and in regard to the relationship between carcinogenesis and mutagenesis of these compounds.

PROPOSED COURSE: Future studies will concentrate on the in vitro activation of aflatoxins using rat organ homogenates. Genetic and biochemical studies will be made in an attempt to identify the active metabolites of aflatoxins.

PUBLICATIONS

Ong, T.: Aflatoxin mutagenesis. Reviews in Genetic Toxicology. Mutation Research 32:35-53, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60039-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Binding of N-acetoxy-2-acetoaminofluorene to DNA of human cells in culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shiu Lan Huang	Senior Staff Fellow	EMB NIEHS
OTHER:	Michael W. Lieberman	Section Head	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

The binding of NA-AAF to DNA in human cultured cells has been studied in order to correlate binding data with information on induced mutation frequency. It was found that, like induced mutation frequency, binding increased with the concentration of the agent in the medium. The data serve to demonstrate the relation between damage and mutations in man.

PROJECT DESCRIPTION

OBJECTIVES: Preliminary experiments in other laboratories indicate that the amount of bound carcinogens or mutagens correlates with the degree of induced mutations and cell transformations in non-human cell systems. In order to understand the mechanisms of mutation, we have carried out experiments to study the binding of NA-AAF to cultured human fibroblasts.

METHODS EMPLOYED: Semi-confluent cells (6×10^6 cell/P150) were used to study binding. Cells were treated with ^{14}C NA-AAF in DMSO at four dosage levels for 60 minutes. The cells were scraped off the culture dishes and the DNA was prepared by CsCl equilibrium centrifugation.

MAJOR FINDINGS: Four doses (10^{-5}, 7.5×10^{-6}, 2.5×10^{-6} and OM) of ^{14}C NA-AAF were used. There was a positive correlation between the amount of NA-AAF bound to DNA and the induced mutation frequency at the HGPRT locus.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Binding studies are of importance in helping to elucidate the mechanism of mutation in man and in establishing the molecular basis for environmental monitoring systems. They are also of potential importance in helping to understand the relation between mutagenesis and carcinogenesis.

PROPOSED COURSE: We propose to continue our analysis of the relation between dosimetry and mutagenesis in cultured mammalian cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60040-01 EMB

PERIOD COVERED
March 15, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Tier One Microsomal Assay for Mutagenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Larry Claxton	Biologist	EMB NIEHS
ASSISTANT PI:	Jack Bend	Visiting Associate	PB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this project are (1) the testing of environmental compounds for mutagenicity in bacteria in the presence of mammalian microsomal enzyme preparations, (2) evaluation of the use of a computerized testing format and data analysis and (3) evaluation of the feasibility of mass screening using the microsomal bacterial assay.

PROJECT DESCRIPTION

OBJECTIVES: The purpose of this project is to evaluate a computerized bacterial microsomal activation system, to test a number of environmental chemicals and to evaluate this system as to its usefulness in mass screening.

METHODS EMPLOYED: Highly trained technicians will follow computerized protocols in the testing of environmental chemicals in a blind study. The data analysis will be done via a computerized system and the feasibility of this system will be evaluated.

MAJOR FINDINGS: The contract has just been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The applicability of computerized testing systems in mutation testing will be determined. Large scale screening in its initial phases will also be evaluated. Environmental chemicals of health and economic importance will be tested.

PROPOSED COURSE: Efforts will be made to initiate this significant project and to evaluate its usefulness.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60041-01 EMB

PERIOD COVERED
October 15, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Synergistic effects of radiation and chemical mutagens on spermatogonia in mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Section Head	EMB NIEHS
	E. R. Soares	Senior Staff Fellow	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Mammalian Genetics & Cytogenetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations using known chemical mutagens have failed to show induction of dominant lethal mutations in spermatogonial stem cells of mice. We are investigating whether pretreatment with irradiation will sensitize spermatogonia to induction of mutations by chemical treatment following the irradiation. Comparisons are being made between groups of animals receiving the combined treatment, radiation alone or only chemical treatment for induction of dominant lethals and chromosome aberrations.

PROJECT DESCRIPTION

OBJECTIVES: Several investigations using known chemical mutagens have failed to show any indication of induction of dominant lethal mutations in the spermatogonial stem cells of mice. Studies with irradiation have shown that delivering a dose in two fractions increased the mutation rate, thus implying that the first fraction caused the stem cells to enter a more sensitive state by the time of the section fraction. It is our purpose to investigate whether pretreatment with irradiation will sensitize spermatogonia to induction of mutations by chemical treatments following the irradiation.

METHODS EMPLOYED: Adult male mice will be irradiated with 300 R of gamma rays. Twenty-four hours after irradiation they will be given a dose of a known potent chemical mutagen (TEM) and thereafter mated in dominant lethal dissection studies. The animals will go through a sterile period caused by the irradiation after which the germ cells tested will correspond to cells in the spermatogonial stem cell stage at the time of treatment. Results will be compared with those of animals receiving only irradiation or only chemical treatment as well as with those of a control group in order to determine any increase in the rate of dominant lethal mutations. Samples of males from all groups will be examined cytologically to determine the rate of induced chromosome aberrations.

MAJOR FINDINGS: The project was initiated during October 1975. Treated animals are presently in the sterile phase.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Mutations occurring in spermatogonial stem cells are of major concern since they not only will deliver large numbers of germ cells carrying identical mutations but will do so for the remainder of the individuals fertile life. Knowledge of the degree of sensitivity of stem cells to mutation induction is pre-requisite to studies intending to estimate the risks from mutagenic agents.

PROPOSED COURSE: Future studies will investigate the effects of presenting chemical mutagens in fraction doses to determine whether there will be a sensitizing effect comparable to pretreatment with irradiation. A cooperative program with biochemists will be entered upon to determine the underlying mechanisms of variation in sensitivity in stem cells

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60042-01 EMB

PERIOD COVERED
February 1, 1976 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Study of Recessive Lethal Mutations Induced in Mice by Chemical Mutagens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Sheridan	Section Head	EMB NIEHS
OTHER:	E. R. Soares	Senior Staff Fellow	EMB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Mammalian Genetics & Cytogenetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the induction of recessive lethal mutations in mice by chemical mutagens are being conducted. Backcross progeny between F_1 offspring to chemical mutagen treated parents and their F_2 daughters are used to detect the presence of recessive lethals. An increase in fetal mortality is used as indicator of the lethal.

PROJECT DESCRIPTION

OBJECTIVES: The induction of recessive lethal mutations by irradiation has previously be demonstrated in the mouse. No adequate study of this class of mutation has been conducted in mice following treatment with chemical mutagens. It is our purpose to investigate whether chemical mutagens will induce recessive lethals in mice and the mode of action and long-term effects of such mutations on mammals.

METHODS EMPLOYED: Inbred male mice of the CBA strain were treated with a known chemical mutagen (TEM) and mated to untreated females of the same strain. F_1 male progeny will be collected and individually mated to females of the strain. A series of backcrosses between F_2 females and their sires will be conducted to determine whether there is an increase in fetal mortality indicative of the presence of a recessive lethal.

MAJOR FINDINGS: F_1 animals are presently being weaned and collected.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It is estimated that on the average the human genome contains 5 deleterious recessive genes. Although there are known examples of genes which have an extreme effect in the homozygote also showing an effect in the heterozygote, for the most part little is known about the mode of action or long-term effects on the barer of such genes. Studies are necessary to determine frequencies of induction of this class of genes by mutagenic agents and their consequences to the individual.

PROPOSED COURSE: Future extensions of these studies will include investigations of the effects of recessive lethals on early developmental stages in homozygotes, comparisons of heterozygotes with normal homozygotes and attempts at biochemical identification of the loci in question.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60043-01 EMB

PERIOD COVERED
October 15, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
A Pharmacokinetic Study of the Distribution of Triethylenemelamine in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. R. Soares	Senior Staff Fellow	EMB NIEHS
OTHER:	H. B. Matthews	Research Chemist	PB NIEHS
	T. E. Eling	Co-Section Head	PB NIEHS
	M. W. Anderson	Co-Section Head	PB NIEHS

COOPERATING UNITS (if any)
Pharmacology Branch, Environmental Biometry Branch

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Mammalian Genetics and Cytogenetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

TEM is a widely used reference chemical mutagen, both as a positive control in routine testing and as the mutagen of choice in the developmental aspects of the mutagenesis programs. The goal of this project is to gain a better understanding of the mode of action of TEM through studies of the pharmacokinetics of this compound.

PROJECT DESCRIPTION

OBJECTIVES: Triethylenemelamine (TEM) is a widely used reference mutagen. In recent studies we have shown the mutagenic effects of TEM to vary with route of delivery. In order that TEM be used as efficiently as possible in routine testing programs and in investigations leading to test development, and in order to gain a more thorough understanding of the action of TEM, we are studying the distribution of TEM in the mouse.

METHODS EMPLOYED: TEM (ethyleneimine-1,2^{14}C) or TEM (triazine-2,4,6^{14}C) was administered by gavage or by i.p. injection to male mice. At one and twenty-four hours after injection, males were killed and various tissues were prepared for oxidation for subsequent analysis. Comparisons of levels of radioactivity in the tissues were made within animals, between compounds, between and within routes of administration and between time periods.

MAJOR FINDINGS: Preliminary findings indicate a rapid turnover and elimination of TEM, regardless of the placement of the label. In addition, it appears from these data that: (1) with oral administration, the ethyleneimine portion of the molecule is cleaved off the triazine ring and subsequently enters the blood in relatively small amounts as compared to i.p. injection, (2) when the label is on the ethyleneimine portion, we have found a retention of the label by specific tissues (i.e., testes and epididymis. This is in contrast to results obtained when the label is present in the triazine ring and (3) although significant differences were observed between routes of administration for ethyleneimine, no such differences were observed when the triazine ring was labeled.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: TEM is a widely used reference mutagen and has been employed as a positive control in numerous tests of various food additives. Furthermore, TEM is often used as the mutagen of choice in the test development aspects of mutagenesis programs. In light of this information it is important that the pharmacokinetics of TEM be studied in order that this reference mutagen be used more effectively in the programs outlined above.

PROPOSED COURSE: Additional studies are planned which will include (1) replication of the preliminary experiments conducted to date and (2) studies which will place further emphasis on the clarification of the observed differences in the distribution and retention of the ethyleneimine moiety versus the triazine moiety.

PUBLICATIONS

Soares, E.R., Eling, T. and Anderson, M.: The Fate and Distribution of TEM in Mice. To be presented at the 7th Annual Meeting of the Environmental Mutagen Society, Atlanta, Georgia, March 11-15, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60044-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Biochemical Genetic Variation: Its Measurement and Significance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Langley	Senior Staff Fellow	EMB NIEHS
OTHER:	Kazuko Ito	Visiting Fellow	EMB NIEHS
	Diana Smith		EBB NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Population Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	1.8	1.8

SUMMARY OF WORK (200 words or less - underline keywords)

Population geneticists have been aware of the enormous genetic variation at biochemical level in natural populations (including humans) for some ten years. No satisfactory interpretation of the biological significance of the subtle variations has been identified. The aim of this project is to discern association among various biochemical polymorphism that might indicate their physiological or developmental effects.

PROJECT DESCRIPTION

OBJECTIVES: The objective of this project is the estimation of association of genetic elements along the chromosome (linkage disequilibrium). To do this we genetically extract second and third chromosome pairs from a natural population of Drosophila melanogaster. This is the only organism in which this is possible. We are also analyzing a large data set from NCSU to estimate the association of allelic variation in enzymes.

METHODS EMPLOYED: Second and third chromosomes were genetically extracted from natural populations of Drosophila melanogaster. These were then genotyped for some 20 enzyme loci and karyotyped.

MAJOR FINDINGS: This work will be completed within the next several months. As of yet the findings cannot be evaluated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: If genetic variation at various enzyme loci were strongly correlated then this would indicate that this polymorphic enzyme variation is biologically significant, i.e., the viability and/or fecundity of an individual is sensitive to the particular allele of each enzyme which he possesses. This in turn would reflect on our evaluation of newly arising mutants (see introduction).

PROPOSED COURSE: This project is coming to an end.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 60045-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mathematical Population Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Guess	Staff Fellow	EBB NIEHS
	J. H. Gillespie	Senior Staff Fellow	EMB NIEHS
	C. H. Langley	Senior Staff Fellow	EMB NIEHS
	G. A. Watterson	Visiting Scientist	EBB NIEHS
	K. S. Crump	Visiting Scientist	EBB NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Population Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to develop a better understanding of environmental effects on gene frequency distributions. The work is conducted in collaboration with scientists in the Environmental Biometry Branch. Current research topics are: (1) spatial dispersion of neutral alleles; (2) the relation between the frequency of a neutral allele and its age; (3) effects of autocorrelated random environments on the progress of natural selection.

PROJECT DESCRIPTION

OBJECTIVES: To work jointly with scientists in EBB on problems in population genetics theory. This work, along with experimental population genetics studies conducted by EBB, is aimed at developing a better understanding of environmental effects on gene frequency distributions.

MAJOR FINDINGS: A model for spatial dispersion of neutral alleles was used in conjunction with Drosophila data to indicate the improbability of the same neutral allele occurring at widely separated localities. Mathematical results needed to study autocorrelated (nonindependent) environmental effects were derived. A well-known problem in the population genetics literature, regarding the theoretical relation between the age of a neutral allele and its frequency, was solved. Results of current research in theoretical population genetics related to the Institute's program were reviewed at a conference sponsored by EMB and EBB.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: An understanding of environmental effects on gene frequency distributions contributes to a knowledge of how genetic variability should be taken into account in predicting long-term effects of environmental changes on public health.

PROPOSED COURSE: Research on effects of autocorrelated random environments will continue. Somewhat less effort is projected since the scientists with whom the joint work was done have left the Institute.

PUBLICATIONS

Crump, K.S. and Gillespie, J.H.: The dispersion of a neutral allele considered as a branching process. J. Appl. Prob. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 60046-01 EMB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Risk Assessment for Environmental Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. G. Hoel	Branch Chief	EBB NIEHS
	H. A. Guess	Staff Fellow	EBB NIEHS
OTHER:	K. S. Crump	Visiting Scientist	EBB NIEHS
	C. H. Langley	Senior Staff Fellow	EMB NIEHS
	P. Y. Chi	Visiting Fellow	EBB NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH
Environmental Mutagenesis Branch

SECTION
Population Genetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop improved statistical techniques for using data from animal carcinogenicity tests conducted at high dose levels to estimate long-term risk from chemical carcinogens at very low dose levels. Existing techniques are evaluated and new techniques are studied, with particular emphasis on those which attempt to relate mechanistically to the carcinogenic process.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: In order to estimate human risk based on animal carcinogenesis experiments, it is currently necessary to extrapolate data from high dose experiments to low doses. The purpose of this project is to study the various mathematical and probabilistic methods for extrapolation with particular emphasis on those which attempt to relate mechanistically to the carcinogenic process.

METHODS EMPLOYED: Various possible models of carcinogenesis were analyzed with respect to low dose kinetics. Considerations of mechanism such as hit models and multistage models were compared to so-called log normal response models. The importance of background incidence and induction time were evaluated. These considerations led to the investigation of "best" risk estimates and optimal experimental designs.

MAJOR FINDINGS: (1) If carcinogenesis by an external agent is considered to be augmenting an already ongoing spontaneous process, then the response will be linear at low dose. (2) If the origin of a cancer is an event in a single cell (as distinct from a whole tissue) then the low dose response will be linear. (3) In general, time of cancer induction must be heavily dependent on dose rate for such consideration to play a role in low dose risk. (4) Linear extrapolation may not always be the conservative technique it has been thought to be. (5) The optimal experimental dose for obtaining the maximal acceptable dose is robust with respect to models when one is extrapolating linearly. (6) A statistical technique for low-dose extrapolation of dichotomous data has been developed mathematically and programmed. The technique is based on a general multistage model, where the number of hits is treated as an unknown to be estimated along with the dose-response coefficients. Best estimates of increased response above background are calculated along with confidence intervals for the estimates.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This project potentially will have an impact upon human risk assessment of environmental carcinogens. This in turn should affect the regulatory process. Further, the techniques developed in this project may be applicable to toxicological effects other than carcinogenesis.

PROPOSED COURSE: (1) Modify the hit models to allow a hit cell to be repaired by some stochastic mechanism and determine if repair can have any significant qualitative effect upon the dose response curve. (2) Consider the implication of variance among individuals in sensitivities to an insult of a carcinogen upon low dose risk estimation. (3) Conduct simulation studies to check the theoretical confidence intervals and goodness-of-fit of the "best" estimates. (4) Study models to estimate time-to-occurrence. (5) Evaluate existing carcinogenesis data for species variabilities. (6) Apply best estimate techniques and existing conservative extrapolation methods to various data sets for comparative purposes.

PUBLICATIONS

Crump, K.S., Hoel, D.G., Langley, C.H. and Peto, R.: Fundamental carcinogenic processes and their implications for low dose risk assessment. Cancer Research (in press).

ENVIRONMENTAL TOXICOLOGY BRANCH

ENVIRONMENTAL TOXICOLOGY BRANCH
Summary Statement

The scientific efforts of the Environmental Toxicology Branch are directed toward the understanding of mechanisms of toxicity. Special emphasis is placed on environmental problems relating to reproduction and development. Toxicologic phenomena are studied from the descriptive to the molecular level in search of a better understanding of mechanisms. This increased understanding contributes to the development of better and more effective test procedures to predict toxicity in humans. Test systems are diverse and range from molecular biochemistry and electron microscopy, through in vitro cell, organ, and embryo cultures, to whole animals. Specific project areas include the following: (1) Environmental Factors Affecting Germ Cell Development; (2) Teratology; (3) Transplacental Toxicology; (4) Molecular Embryology; (5) Perinatal Enzymology and Endocrinology; (6) Chronic Organ Toxicity; (7) Toxicological Test Systems Development; and (8) Effects of Environmental Agents on Learning and Behavior.

ENVIRONMENTAL FACTORS AFFECTING GERM CELL DEVELOPMENT

Dr. Lee has been investigating whether the DNA repair system in mouse germ cells can be utilized as a predictive test system for environmental mutagens. Biochemical and toxic effects of environmental agents on mouse spermatogenesis are also being studied. Enzyme development during spermatogenesis is being determined in an attempt to define "marker" enzymes indicative of normal and abnormal cellular differentiation. The interrelationship between hormone action and ontogeny of testicular enzyme synthesis is also being considered as well as the nature of the blood-testis barrier.

Spermatogenic Cell Enzymes. Postnatal development of testicular enzymes associated with germ cell differentiation in CD-1 mice was studied. Eight enzymes were selected: hyaluronidase (H), lactate dehydrogenase isoenzyme-X (LDH-X), and dehydrogenases of sorbitol (SDH), α-glycerophosphate (GPDH), glucose-6-phosphate (GPDH), malate (MDH), glyceraldehyde-3-phosphate (G3PDH), and isocitrate (ICDH). Enzyme activities in testicular homogenates were determined by kinetic assays using spectrophotometry. Two types of enzyme developmental patterns were observed. One was represented by H, LDH-X, SDH, and GPDH; and the other, by G6PDH, MDH, G3PDH, and ICDH. The former was characterized by a change in enzyme activities from low in newborn to high in adult, while in the latter this development was reversed with the activity of two complementary enzyme systems crossing each other at puberty. The distribution of SDH and G6PDH in epididymis and vas deferens was also studied. Epididymis and vas

deferens of adult mice were high in SDH and low in G6PDH, whereas the reverse was true in newborn mice. Histological observation indicated a close correlation between the sequential changes in enzyme activities and stages of spermatogenic cell differentiation.

Blood-Testis Barrier. Physiological studies have described the existence of a blood-testis barrier (BTB) surrounding the seminiferous tubules of the mammalian testis. Studies were initiated to better define the role of the BTB with regard to the penetration of exogenous chemicals to male germ cells. Permeability of nonelectrolytes of various molecular sizes, acidic compounds with varying partition coefficients and pK_a values, (such as salicylic acid, barbiturates, and sulfonamides) across the BTB were studied. Permeability of nonelectrolytes was demonstrated to be dependent upon their molecular size, suggesting bulk flow through water-filled pores. On the other hand, permeability of acidic drugs with varying pK_a values depended upon their partition coefficients. Transport of these chemicals from blood to seminiferous tubules closely resembled their transport from blood to cerebrospinal fluid. It appears that the BTB is a complex multicellular system composed of membranes surrounding the seminiferous tubules and the several layers of spermatogenic cells organized within the tubules which restricts the permeability to the male germ cells of many foreign compounds.

TERATOLOGY

The Teratology group is concerned with the etiology of birth defects in mammals, the development of improved test methods for assessing the teratogenic potential of environmental agents, and the delineation of how teratogens alter the course of development.

Dr. Staples and coworkers have been studying the effects of test agents administered directly to the conceptus, rather than to the dam are being studied as a possible means of minimizing differences in toxic responses among laboratory species. Qualitative and quantitative comparisons of responses by the conceptus are made. DMSO, captan, and thalidomide have been administered to dams, injected between implantation sites *in utero*, added to preimplantation embryos cultured *in vitro*, and injected directly into blastocysts. Attempts are also being made to determine the extent of absorption of the test agent by the embryo and its biological half-life.

Offspring with cleft palates have been detected after administration of the progestational steroid medroxyprogesterone acetate (MPA, Provera®) to pregnant rabbits. Teratogenicity was not noted in rats or mice even at the maximum tolerated dose. In view of this obvious species difference and the rare occurrence of palatal malformations in the rabbit, the teratogenic mechanisms of MPA are being explored further in rabbits.

Potential chemical interactions in our complex environment are multiple, hence synergistic responses might explain a large part of the still undefined incidence of birth abnormalities. Chemicals which appear innocuous using currently accepted laboratory tests might actually be toxic in combination with other chemical, physical, physiologic, or pathologic factors. Dichlorvos (a widely used insecticide) was studied in three species by several routes alone and in

combination with other agents (e.g., alcohol, carbon monoxide, hypothyroidism). This work was conducted jointly through a contract with Dow Chemical Company and intramural efforts. Current investigations focus on pollutants of our atmosphere (e.g., SO_2) and drinking water (e.g., chloroform).

As part of the US-USSR Cooperative Program, Dr. Staples and his coworkers studied the teratogenic potential of two insecticides (Dipterex, Imidan) in the rat. A positive teratogenic response was obtained with Dipterex but not with Imidan. Dipterex is now being studied in additional species.

The world's literature on the teratogenic effect of environmental agents is being organized into a teratology information program that will be available to scientists and to physicians and which will aid in determining priorities and planning future investigation.

Dr. Maurer, in collaboration with scientists in Germany, also have been studying uterine luminal fluid proteins in rabbits to determine their role during early pregnancy and their susceptibility to change in an altered environment. For instance, injection of progesterone before ovulation produces a premature appearance of uteroglobin, a uterine luminal protein, thereby altering the synchrony between uterine environment and the developing embryo.

TRANSPLACENTAL TOXICOLOGY

Dr. McLachlan and coworkers have been studying the effects of environmental agents on reproductive tract function using mice. Current studies involve the synthetic estrogen, diethylstilbestrol (DES), a common environmental chemical currently used as a livestock food additive and gynecologic medication. Experiments in their laboratory have demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the female offspring. This effect is dose-related and is due, in part, to the relative inability of the female offspring to ovulate. The reproductive tracts in the prenatally, drug-exposed offspring have neoplastic lesions which include changes resembling vaginal and/or uterine adenocarcinoma. Likewise, 60% of the male offspring are sterile following prenatal exposure to DES. The most significant male genital tract lesions were epididymal cysts, prostatic inflammation, and nodular masses in the area of the prostate. These results may be pertinent to the development of an animal model to study the reproductive tract lesions in humans whose mothers had been given DES during pregnancy. This is reinforced by recent reports of epididymal cysts, prostatic inflammation, azoospermia, and abnormal sperm morphology in young men whose mothers were treated with DES during their gestations. The pathogenesis of the genital lesions in male, as well as female offspring, is being studied with special emphasis on the embryonic tissues involved. Further studies are underway on the mechanisms of DES-induced subfertility in mice exposed prenatally to this compound.

Since little is known concerning the physiologic disposition and metabolism of DES during gestation, the fate of radiolabeled DES in the pregnant mouse was studied. The compound is rapidly cleared from the maternal plasma following intravenous administration. Relative to the plasma, DES accumulates in the maternal liver and, to a lesser extent, in the uterus and fetus. The activity of glucuronyltransferase in the maternal and fetal liver helps account for the

accumulation of radioactivity identified as DES conjugates in these two organs. Developmental studies showed that hepatic glucuronyltransferase activity towards DES arises very early in fetal life. Moreover, radioactivity associated with DES was shown to accumulate in the reproductive tract of the fetus after administration of DES to the mother. Current research includes the metabolism of DES and analogs as well as their interaction with cellular macromolecules.

Attempts are being made to develop and utilize organ and cell culture systems to study direct fetal reproductive tract effects of DES and related chemicals. For example, work is continuing on the development of in vitro mammalian tissue and organ culture test systems which can be utilized for rapid prediction of potentially toxic environmental agents. Progress has been made regarding development of a viable tissue or organ culture system of mammalian gonadal tissue. The mechanisms by which chemical agents affect oogenesis and subsequent stages of oocyte maturation and follicular development will be studied using this system. Oocyte maturation in ovary explants taken from 16-day old embryos was followed for 12-14 days in culture. Oocyte maturation in vitro occurred in a manner analogous to maturation in vivo to about 25 days postconception. Effects of temperature and alterations in growth medium on follicular maturation have been established. Attempts to establish consistent and reproducible stages of growth and maturation of fetal and prepubertal ovarian explants will be continued.

Dr. Jurgelsky has continued his studies to evaluate the marsupial as a model for carcinogenesis. The autopsy phase of this project has been completed. Ethylnitrosourea, a known potent carcinogen, was administered orally as a single dose (100 mg/kg) to opossums at ten different stages of development. Eye tumors and several other unusual tumors have been found in ENU-treated animals.

PERINATAL ENZYMOLOGY AND ENDOCRINOLOGY

Research efforts of this group are concerned with perinatal aspects of enzymology, endocrinology, and pharmacokinetics. There is particular interest in characterizing the roles that alterations in developmental enzyme patterns, hormone metabolism and binding, placental transfer, and xenobiotic biotransformation play in reproductive and developmental toxicity. Dr. Lucier and coworkers are studying perinatal metabolism and binding of xenobiotics and hormones. The relative rates of UDP glucuronyltransferase and β-glucuronidase were studied during perinatal development in hepatic and extrahepatic tissues to determine the balance between glucuronidation or deglucuronidation at different developmental stages. In general, deglucuronidation predominated over glucuronidation in fetal tissues whereas the converse was evident in adults. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), an extremely toxic contaminant of some organochlorine compounds, was shown to be a potent inducer of some hepatic and extrahepatic drug-metabolizing enzymes. TCDD, administered during gestation, induced the postnatal activities of p-nitrophenol glucuronyltransferase and benzypyrene hydroxylase in rats. Foster mother experiments revealed that the postnatal induction was caused primarily by newborn exposure to TCDD in the mother's milk. Tissue distribution experiments with TCDD-^{14}C confirmed these findings. Although TCDD induced nonsteroid glucuronidation, no significant effects were evident on the postnatal development of DES glucuronidation which,

like the steroid pathway, was not affected by gestational TCDD treatment. The fetal distribution of DES and DES-glucuronide, at different stages of development correlate well with the perinatal development of steroid glucuronyltransferase activity. Although the mouse placenta appeared to retard the passage of DES into the fetal compartment, a three-fold accumulation (relative to fetal plasma) was found in the fetal reproductive tract. Placental transfer rates of cadmium in rats increased significantly with increasing gestational age and increasing dose. Higher percentages of administered cadmium were detected in fetal tissues as the dose was increased from 0.1 to 1.6 mg/kg. Liver was the major fetal accumulation site. Current research involves placental transfer of various PCB isomers, and the effect of these hydrocarbons on neonatal programming of steroid metabolism and the ontogeny of steroid receptors.

Dr. Schiller is studying the development of active transport processes in the mammalian small intestine. The initial focus has been on the development of metabolic energy-producing processes as they relate to the onset of active transport of nutrients. Of particular interest has been the mechanisms by which an animal converts from anaerobic to aerobic metabolism at birth. The suggestion that high aerobic glycolysis observed in intestinal tissue (which is also a characteristic of tumor tissue metabolism) resulted from deficient mitochondrial transport of reducing equivalents was examined. Both the permeability characteristics of the isolated intestinal mitochondria and the enzymic constituents of this tissue indicate that the α-glycerol phosphate and malate/aspartate substrate cycles do function in the adult and may play an important role in the energy metabolism of this organ. The presence of each of the enzymic constituents of these cycles in the appropriate subcellular fractions of the intestine was established in the hamster from the thirteenth day of gestation, through the perinatal period, to the adult stage. The cycle enzymes monitored were flavoprotein-L-glycerol 3-phosphate dehydrogenase, NAD^+-L-glycerol 3-phosphate dehydrogenase, L-malate dehydrogenase, and L-aspartate aminotransferase as well as the marker enzymes lactate dehydrogenase and citrate synthetase. The developmental pattern of intestinal pyruvate dehydrogenase suggests that this enzyme is important to the perinatal metabolic transition period. The activation of the phosphorylated form of this enzyme by a change in the redox state of this tissue at birth might be particularly important. Additional studies have utilized isolated intestinal epithelial cells to examine the susceptibility of the different cell types to induction of mixed function oxidases by xenobiotics. Future projects will include the use of isolated cells to study the transport of nutrients and xenobiotics in culture.

CHRONIC ORGAN TOXICITY AND TEST SYSTEMS DEVELOPMENT

This group emphasizes biochemical toxicology in a coordinated effort to determine basic biochemical and physiological mechanisms through which environmental agents exert their toxic effects in mammalian tissues and to apply this information to the design of rapid and effective test procedures to predict toxicity in man.

Dr. Woods and coworkers have been studying factors affecting hepatic heme synthesis and its utilization during mammalian development. Differences in the regulatory and biochemical properties of several of the heme biosynthetic pathway enzymes in adult and fetal liver, including those of δ-aminolevulinic

acid (ALA) synthetase which is considered to be rate-limiting in this process, have been elucidated. These studies have also shown that heme plays an important role in regulating the synthesis of various mitochondrial proteins in fetal mammalian liver, an action which is probably mediated through a direct stimulatory effect of heme on the synthesis of apocytochrome oxidase and, perhaps, other hemoproteins which comprise integral portions of the mitochondrial structure. Because of the important regulatory role played by heme during fetal hepatic development, it is likely that late gestation may be a period of enhanced susceptibility to the effects of environmental agents which selectively interfere with heme biosynthesis or its utilization. Studies are currently in progress to assess the effects of prenatal exposure to hematotoxic agents on the postnatal development of hepatic hemoprotein function.

Drs. Woods and Fowler are attempting to define early ultrastructural and biochemical effects of heavy metals and other environmental agents on mammalian tissues, as a means toward providing insight into the mechanisms of toxicity of these substances, as well as deriving early warning tests for predicting sub-toxic exposure to such agents. These studies include correlation of subcellular morphologic observations with biochemical alterations in tissues of animals chronically exposed to low levels of toxic agents for various time periods. These studies also attempt to define the ability of electron microscopy to predict early signs of chronic toxicity in mammals. In current studies, the toxicity of arsenic, cadmium, bismuth, methyl and inorganic mercury, dioxin (TCDD), and vinyl chloride are being studied. Results of these experiments indicate that morphological observations of mitochondrial swelling and changes in the size and volume density of these organelles can be directly correlated with specific alterations in the activities of mitochondrial membranal enzymes. Such alterations produce enzyme profiles which characterize exposure to a particular environmental agent. Studies are currently in progress to utilize such profiles to develop test procedures which will permit identification of preclinical exposure to specific environmental substances in a multi-element environment.

Modifications of plasma membranal structure and function during development which might affect fetal responsiveness to teratogenic and other toxic agents are being investigated by Dr. Wilson. Inherent in these studies is a characterization of several plasma membranal enzymes, including prostaglandin synthetase, $(Na+K^{+})$ ATPase, and adenyl cyclase, during development, and an investigation of the role such enzymes play in overall growth and function of the fetus. Studies currently underway suggest that patterns of development of plasma membranal enzymes in feto-placental tissues may be closely correlated with embryonic differentiation. Thus, the behavior of these enzymes during gestation is likely to play a significant role in determining responsiveness of embryonic cells to teratogenic stresses such as nutritional deficiencies, drugs, and other environmental agents.

EFFECTS OF ENVIRONMENTAL AGENTS ON LEARNING AND BEHAVIOR

A program of behavioral toxicology is being developed at the NIEHS. This program will attempt to define methods to determine what environmental stresses produce behavioral alterations in mammals and lower species. Model test systems which stimulate and predict behavioral toxicity in humans will be developed

and neurochemical mechanisms of behavioral toxicity considered. Dr. Kimmel's research involves the use of lead as a prototype compound for assessing the utility of many different behavioral tests which are currently in use. At high levels of lead (50 and 250 ppm in drinking water) given pre- and postnatally there are effects on body weight gain, delays in physical developmental landmarks (e.g., vaginal opening), and in the ontogeny of reflex behaviors, but no effects on morphologic development during pregnancy. Further studies will provide information on the effects of low levels of lead (0.5 and 5 ppm) on more complex behaviors such as learning and memory and sexual behavior. The results of the behavioral assessment will be used to develop a battery of tests for use in screening compounds with suspected neurotoxic potential.

In the coming year, the Environmental Toxicology Branch will continue to expand its research activities in each of the areas described and a more systematic routine testing capability will be developed. Test methods will be validated and improved, and efforts made to extend laboratory animal observations to the human population. An increasingly strong interaction between laboratory scientists and clinicians and epidemiologists will be developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70020-04 ETB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effect of Environmental Agents on Ontogenesis After Parental Exposure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Staples	Research Physiologist	ETB NIEHS
OTHER:	R. R. Maurer	Senior Staff Fellow	ETB NIEHS
	M. H. Lu	Visiting Fellow	ETB NIEHS
	U. Schneider	Visiting Fellow	ETB NIEHS

COOPERATING UNITS (if any)

Research Triangle Institute (Procurement Contract: NIH-74-C-390)

LAB/BRANCH

Environmental Toxicology Branch

SECTION

Teratology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.65	1.45	1.20

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to estimate the potential hazard of selected environmental agents to human development. Studies were conducted to determine the teratogenic potential of Dipterex, captan, ethylenethiourea, asbestos, noise, and Provera in laboratory animals (mice, rats, rabbits, or hamsters).

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To determine the developmental toxicity and teratogenic potential of single or combined environmental components in mammals by application of the agents to the parent(s) before mating or to the dam during gestation.

METHODS EMPLOYED: Test agents were given to pregnant females of four species: mouse, rat, hamster, and rabbit by two or more routes, e.g., per os, subcutaneous, intrauterine, for various periods of ontogenesis. Doses were selected on the basis of preliminary study. Those which produced a given pharmacological response (maximum tolerated dose, comparable blood levels, 50% fetal mortality, etc.) were selected. Stresses or disease states that may be elicited before mating were also studied for teratogenic potential either alone or in combination with other environmental components. An attempt was made to compare the sensitivity of various approaches. Qualitative or quantitative differences or similarities in teratogenic response were noted. Resulting offspring were examined for alterations in the incidence of external, visceral, and skeletal changes shortly before term.

MAJOR FINDINGS: Dipterex, found to be teratogenic in the rat in FY 1975, was given to hamsters and mice by gavage in divided daily doses to determine its teratogenic potential in these species. Teratogenic activity was less in the hamster than in the rat and was weaker still in the mouse. The spectrum of malformations seen was similar in both the rat and hamster.

Asbestos (Chrysotile) fibers were suspended in filtered tap water (up to 143 µg/ml) which was offered to mice as drinking water from Days 1 through 15 of gestation. No adverse effects were seen in reproductive parameters or in development of the fetuses removed from dams that consumed a maximum total dosage of 15.4 mg asbestos/female.

Surgical thyroparathyroidectomy of female rats during the first 3 weeks after birth, a critical period for development of the central nervous system, resulted in reduced weight gain, delayed maturation, reduced fertility, and increased maternal mortality before parturition. Maternal mortality was often preceded on Days 7 and/or 8 of gestation by cage circling, wry neck, and hyperirritability. Dams surviving to scheduled sacrifice had fewer fetuses than sham-operated dams, and fetal maturation was retarded.

Female mice that drank NIEHS tap water during pregnancy were found to have significantly more fetal loss than those drinking charcoal filtered deionized and distilled water.

A manuscript on the medroxyprogesterone (Provera) study was submitted for publication, and another is being prepared. Manuscripts are being prepared also on the effects of thyroidectomy, ethylenethiourea, dichlorvos, and asbestos.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Through the efforts described, the teratogenic potential of environmental components is evaluated. The teratogenic response of various species to some of the agents being tested *in vivo* is compared to the response after exposure of preimplantation embryos during culture. Our hope is that a means of testing can be derived that will improve extrapolation of experimental results to man. Modern man is exposed continuously to a multitude of physical stresses and chemical insults, yet most agents tested for teratogenic potential are still being studied as single entities even though synergistic effects in animals have been documented. One of the goals of this study is to determine the likelihood of synergism of teratogenic potential with combinations of environmental components to which the housewife might reasonably be exposed. Environmental components to be tested are first studied individually and then, depending upon the information gained, combinations of components are tested.

PROPOSED COURSE: Environmental agents identified as high priority for NIEHS study by an Advisory Committee will be tested for teratogenic potential. In addition, agents that appear as pollutants in chlorinated drinking water are to be tested, e.g., chloroform is currently under investigation.

PUBLICATIONS

Staples, R. E., Kellan, R. G., and Haseman, J. K.: Developmental toxicity in the rat after ingestion or gavage of organophosphate pesticides (Dipterex, Imidan) during pregnancy. Environ. Health Persp. 13: 133-140, 1976.

Kimmel, C. A., Cook, R. O., and Staples, R. E.: Teratogenic potential of noise in mice and rats. Toxicol. Appl. Pharmacol. (in press).

McKinney, J. D., Maurer, R. R., Hass, J. R., and Thomas, R. O.: Possible factors in the drinking water of laboratory animals causing reproductive failure. Proceedings of the Symposium on Identification and Analysis of Organic Pollutants in Waters, First Chemical Congress of North American Continent, Mexico City, Mexico (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70021-04 ETB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of Mammalian Embryos After Direct Exposure of Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Staples	Research Physiologist	ETB	NIEHS
	R. R. Maurer	Senior Staff Fellow	ETB	NIEHS
OTHER:	U. Schneider	Visiting Fellow	ETB	NIEHS

COOPERATING UNITS (if any)

Research Triangle Institute (Procurement Contract: NIH-74-C-390)

LAB/BRANCH

Environmental Toxicology Branch

SECTION

Teratology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.45	0.65	0.80

SUMMARY OF WORK (200 words or less - underline keywords)

This work was undertaken to determine whether direct application of test agents to embryos followed by continued development to near term in vivo would prove to be a valuable approach for detection of teratogenicity and embryotoxicity. In FY 76 this approach was used in mice for testing of asbestos, chloroform, and captan.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To determine the effects of various chemical and physical entities, acting alone and in combination, on subsequent development of mammalian gametes and embryos after in vitro or intrauterine exposure.

METHODS EMPLOYED: Embryos are recovered from donor animals and cultured in vitro for varying periods. Chemicals or conditions being tested are generally applied during the culture period. Some preimplantation embryos are transferred to recipient females while others are examined for morphologic or chemical changes. Transferred embryos are usually examined near term for external, visceral, and skeletal malformations, but when indicated, they may be studied at earlier stages of gestation or postnatally. Radiolabeled test chemicals are used to determine uptake and distribution in embryonic tissues.

MAJOR FINDINGS: Asbestos fibers were not shown to penetrate the zona pellucida of mouse blastocysts after in vitro exposure during the preimplantation period but survival of embryos exposed to asbestos was decreased. Removal of the zona pellucida permitted penetration of asbestos fibers into the blastocyst cells as demonstrated by electron microscopy. Since the fibers were found in cytoplasmic vacuoles, it is considered likely that uptake was by phagocytosis. Development of a few zonaless blastocysts, after culture with asbestos in the medium and transfer to pseudopregnant females, did not appear to be adversely affected by exposure to asbestos. A manuscript presenting these data is in internal review.

Captan was administered to rabbits by intrauterine injection on Day 10 of gestation at dosages of 30, 300, and 3000 mg/uterine horn. In contrast to the previous findings in mice, the administration of captan did not appear to have an adverse effect on fetal development.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Techniques permitting development of preimplantation embryos in vitro with retention of viability to term after transfer to the uteri of recipient females and after application in utero makes possible direct exposure of mammalian embryos to test agents. This permits investigators to distinguish between direct and indirect effects of environmental insults on embryo development. Results of local exposure are compared to those seen following administration to the dam or to the uterus. Perhaps variability of response to teratogens can be reduced among species if maternal pharmacokinetics are circumvented. Teratogenic agents might also be identified after local application of only a small fraction of the dose currently required by in vivo tests.

PROPOSED COURSE: To publish the data collected previously on captan and thalidomide and to continue studies initiated on pollutants of chlorinated drinking water, e.g., chloroform.

PUBLICATIONS

Staples, R. E.: Potential of direct application techniques for detection of teratogens. In: New Approaches to the Evaluation of Abnormal Embryonic Development. Proceedings of 2nd Symposium on Prenatal Development, organized by Sonderforschungsbereich 29 "Embryonal-Pharmacologie" W. Berlin, Germany, September 12-16, 1975 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70040-05 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Low Temperature Storage of Mammalian Embryos

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. R. Maurer	Senior Staff Fellow	ETB	NIEHS
OTHER:	U. Schneider	Visiting Fellow	ETB	NIEHS
	J. K. Haseman	Mathematical Statistician	EBB	NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Teratology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Factors important to maintain viability of frozen and thawed mammalian embryos were identified. Optimal levels of factors needed to maintain embryo viability differed for stage of embryo development, strain, and species. Rabbit and mouse embryos were frozen and stored at -196°C. Viable young of both species were obtained after thawing and transfer of the embryos to foster dams.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: (1) To establish methodology for the storage of viable mammalian embryos; (2) to assess the effects of freezing and low temperature storage on subsequent *in vitro* and *in vivo* development; (3) to determine if mammalian embryos at various preimplantation stages of development respond similarly to freezing and low temperature storage; and (4) to provide a bank of stored embryos.

METHODS EMPLOYED: Embryos are recovered by conventional methods from mice on Day 3 or 4 of gestation and from rabbits on Day 2 or 3 of gestation. The embryos are washed with and placed in a phosphate buffered saline solution fortified with pyruvate, bovine serum albumin, and DMSO. Embryos are frozen slowly (0.5 to 1.5°C/min), and the temperature gradually reduced to -100°C before the embryos are placed in liquid nitrogen (-196°C). After various periods of storage, the embryos are thawed slowly (4 to 50°C/min), washed, and placed in culture for 48 hours. After culturing, the resulting mouse blastocysts are transferred to the uterus of Day 3 recipient mice. Some of the recipients are allowed to deliver. The fetuses from the remaining recipients are removed by Cesarean section one or two days before parturition and examined for teratological changes. Rabbit morula are transferred immediately after thawing to Day 2 recipients for subsequent evaluation of *in vivo* development.

MAJOR FINDINGS: Optimization of conditions for freezing 8-celled and blastocyst stage mouse embryos demonstrated that DMSO concentration and equilibration were of utmost importance. At least 80% of the frozen embryos retained viability in culture after thawing, and upon transfer to pseudopregnant females, about one-third developed into viable fetuses. A manuscript, which indicates that fetuses developed from frozen embryos were without structural alterations and post-partum survival, growth, and fertility were similar to control animals, was submitted to Biology of Reproduction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Human spermatozoa are frozen and banked for routine clinical inseminations as well as for vasectomized males who may at a later time desire children. The eventual storage of human embryos must also be considered. The main cryoprotective agent is DMSO, which is known to be teratogenic at high levels. Therefore, the potential hazard should be assessed through use of animal models.

The freezing and storage of embryos can aid experimentation by providing a readily available source of material, preserving mutant strains of species, and providing a more precise measurement of genetic drift as well as changes in susceptibility to various environmental insults in mammalian species and strains. Proper preservation of embryos will reduce preparation artifacts, thereby aiding subcellular studies. The general technique being developed can also provide further information regarding the freezing and banking of human tissues and organs.

PROPOSED COURSE: To complete ongoing studies and to publish the results.

PUBLICATIONS

Bank, H. and Maurer, R. R.: Cryogenic preservation of eight celled and morula staged rabbit embryos. In O. Muhlbock (Ed.): Proceedings of the Workshop on Basic Aspects of Freeze Preservation of Mouse Strains. Gustav Fisher Verlag. Stuttgart, 1975, pp. 35-43.

Maurer, R. R. and Haseman, J. K.: Freezing morula stage rabbit embryos. Biol. Reprod. (in press).

Maurer, R. R.: Storage of mammalian oocytes and embryos: A review. Can. J. Anim. Sci. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70041-01 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Uterine Luminal Proteins and Embryonic Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. R. Maurer	Senior Staff Fellow	ETB NIEHS
OTHER:	H. M. Beier	Visiting Associate	ETB NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Teratology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The biologic significance of changes in uterine luminal proteins was investigated. Protein fractions did not stimulate 8-celled rabbit embryos, but permitted morulae to develop in vitro. Blastocysts which developed blastocoeles either in vitro or in vivo showed immediate radial expansion when placed in medium supplemented with nonfractionated uterine proteins; whereas, those cultured in bovine serum albumin supplemented medium tended to "hatch" and the blastocyst coverings did not thin or expand. This study demonstrates that synchrony between the embryo and the uterine environment is necessary to permit normal embryonic development.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the biologic significance of uterine luminal proteins present during early pregnancy and (2) to determine whether quantitative or qualitative changes in luminal secretions occur when animals are placed under stress or subjected to environmental agents.

METHODS EMPLOYED: The effects of uterine luminal proteins on embryo development *in vitro* were studied. The effects of proteins isolated from pseudopregnant and pregnant (5-6 days) rabbit were compared to bovine serum albumin which is normally a part of the medium used to culture preimplantation stage embryos. Protein concentration was determined using the Lowry method. Qualitative protein changes were examined electrophoretically.

MAJOR FINDINGS: We reported previously that fractionated uterine proteins did not stimulate development of 8-celled rabbit embryos but permitted morulae to develop *in vitro*. Using nonfractionated uterine proteins, embryonic growth of 8- to 16-celled embryos, morulae, and Day 4 blastocysts was stimulated *in vitro*. Embryos developing blastocoeles *in vitro* expanded radially in a manner similar to that seen *in utero* when supplemented with nonfractionated protein containing media. In contrast, embryos cultured in bovine serum albumin supplemented medium expanded slower and tended to "hatch" out of the blastocyst covering. Densitometric tracings of the disc gel separations of the nonfractionated uterine protein showed that the main components were albumin and uteroglobin with a small β-glycoprotein peak. A manuscript presenting these data was submitted for publication to the Journal of Reproduction and Fertility.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Elucidation of the biologic significance of uterine luminal proteins would add to our knowledge of the process necessary for the further development of preimplantation embryos. Environmental components such as DDT and nicotine appear to be associated with and concentrated by the uterine luminal proteins. Both DDT and nicotine have also been shown to affect fetal development. Additional experimental effort may demonstrate that other environmental agents can alter uterine luminal proteins and thereby affect embryonic development. These findings might be of importance in population control.

PROPOSED COURSE: To complete studies in progress and to publish the results.

PUBLICATIONS

Beier, H. M. and Maurer, R. R.: Uteroglobin and other proteins in rabbit blastocyst fluid after development *in vivo* and *in vitro*. Cell Tiss. Res. 159: 1-10, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70060-03 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effect of Prenatal Exposure to Foreign Chemicals on Genital Tract Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. McLachlan	Research Associate	ETB NIEHS
OTHER:	H. C. Shah	Visiting Fellow	ETB NIEHS
	G. W. Lucier	Head, Devel. Tox. Group	ETB NIEHS
	L. Levy	Research Chemist	EBCB NIEHS
	M. Hogan	Mathematical Statistician	EBB NIEHS
	B. C. Bullock	Prof., Comparative Med.	Bowman-Gray School of Medicine

COOPERATING UNITS (if any)
Bowman-Gray School of Medicine
Environmental Biology and Chemistry Branch, NIEHS
Environmental Biometry Branch, NIEHS

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Transplacental Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	1.5	1.9

SUMMARY OF WORK (200 words or less - underline keywords)

The long range goal of this project is to evaluate the effects of prenatal exposure to foreign chemicals on the development of reproductive tract function in mammals. Total reproductive capacity of the prenatally, drug-exposed females is assessed by repetitive forced breeding methods. These techniques are coupled with the determination of ovarian periodicity by daily vaginal smears, superovulatory challenges with exogenous gonadotropins, and collection and evaluation of fresh ova. Histopathologic evaluations of the ovary, including quantitative determinations of the various follicular classes to evaluate the functional status of the female gonad, are also performed. Biochemical, physiologic, and morphologic procedures are also used which include electrophoresis, histochemistry, autoradiography, and microdissection. Studies will evaluate the modification of cytoplasmic steroid hormone receptors in the reproductive tracts of drug treated animals. The metabolism, distribution, and selected chemicobiological interactions of test compounds are studied. The transplacental carcinogenic potential of the test compounds is determined by pathologic evaluations of the offspring at appropriate times in adult life. Finally, the effects of environmental agents on the process of oogenesis will be studied in vitro.

PROJECT DESCRIPTION

OBJECTIVES: (1) To evaluate the effects of prenatal exposure to environmental chemicals on the subsequent reproductive capacity of the offspring; (2) to investigate the mechanisms involved in the production of subfertility in mammals as a result of their in utero exposure to foreign chemicals; (3) to assess the transplacental carcinogenic potential of these compounds; (4) to study the physiologic disposition and metabolism of these compounds in the pregnant animal and fetus; (5) to study chemico-biological interactions of transplacental toxicants, with special emphasis on structure-activity relationships; (6) to determine if prenatal exposure to environmental agents can alter the cytoplasmic receptors for steroid hormones in reproductive tract tissue; (7) to develop and utilize organ culture systems to study the effects of environmental chemicals on the fetal ovary and reproductive tract in vitro; and (8) evaluate the above animal models as predictors of human response.

METHODS EMPLOYED: The most sensitive measurement of female gonadal function is her total reproductive capacity as determined by repetitive forced breeding techniques. In order to assess the functional status of the female gonad, these techniques were coupled with the determination of ovarian periodicity, quantity, and morphology of ova obtained from the female reproductive tract, and histopathologic evaluations of the ovary including quantitative determinations of the various follicular classes. Biochemical, physiologic, and morphologic procedures were used which included polyacrylamide gel electrophoresis, radioimmunoassay, histochemistry, autoradiography, inverse isotope dilution analysis, and microdissection. Organ culture techniques were developed to maintain explants of fetal ovaries and reproductive tracts.

MAJOR FINDINGS: The synthetic estrogen, diethylstilbestrol (DES), is a common environmental chemical currently used as a livestock food additive and gynecologic medication. Experiments in our laboratory have demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the female offspring. This effect is dose-related and is due, in part, to the relative inability of the female offspring to ovulate. The reproductive tracts in the prenatally, drug-exposed offspring have neoplastic lesions which include changes resembling vaginal and/or uterine adenocarcinoma. Likewise, 60% of the male offspring are sterile following prenatal exposure to DES. The most significant male genital tract lesions were epididymal cysts, prostatic inflammation, and nodular masses in the area of the prostate. These results may be pertinent to the development of an animal model to study the reproductive tract lesions in humans whose mothers had been given DES during pregnancy. This is reinforced by recent reports of epididymal cysts, prostatic inflammation, azoospermia, and abnormal sperm morphology in young men whose mothers were treated with DES during their gestations.

Since little is known concerning the physiologic disposition and metabolism of DES during gestation, the fate of radiolabeled DES in the pregnant mouse was studied. The compound is rapidly cleared from the maternal plasma following intravenous administration. Relative to the plasma, DES accumulates in the

maternal liver and, to a lesser extent, in the uterus and fetus. The activity of glucuronyltransferase in the maternal and fetal liver helps account for the accumulation of radioactivity identified as DES conjugates in these two organs. Developmental studies showed that hepatic glucuronyltransferase activity towards DES arises very early in fetal life. Moreover, radioactivity associated with DES was shown to accumulate in the reproductive tract of the fetus after administration of DES to the mother.

Attempts are being made to develop and utilize organ and cell culture systems to study direct fetal reproductive tract effects of DES and related chemicals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Although many compounds are continuously introduced into our environment, few of them have been examined for their potentially toxic effect on reproduction and development. Virtually nothing is known about the effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring. The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such *in utero* drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. Given the possibility of long-term genetic damage to the developing oocyte or transplacental carcinogenetic changes in the reproductive tract, reduced fertility in the offspring may be only the most obvious consequence of prenatal exposure to environmental chemicals.

PROPOSED COURSE: The studies dealing with the total reproductive capacity of mice exposed to diethylstilbestrol *in utero* will be continued with emphasis placed on investigating the mechanisms underlying these observations. The pathogenesis and long-term outcome of the neoplastic changes in the offspring's genital tract will be evaluated; attempts will be made to establish biochemical and/or morphologic markers for these changes. Experiments to determine sensitive periods and structure-activity relationships involved in these effects will be continued. Further studies will be done on the metabolism and distribution of DES and related chemicals with special emphasis on the biological significance of potentially activated metabolites; these studies should aid in generalizations to other chemicals in this class. Moreover, studies of steroid receptor proteins in prenatally DES-exposed females are being continued. The mouse fetal gonad organ culture system will be refined and expanded to include co-culturing of fetal gonads and genital tract tissue. Tests of extrapolations from animal studies to humans will be attempted.

PUBLICATIONS

McLachlan, J. A., Newbold, R. R., and Bullock, B.: Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. *Science* 190: 991-992, 1975.

McLachlan, J. A.: Lesions in the genital tract of male mice following prenatal exposure to diethylstilbestrol. Proceedings of the 17th Annual Meeting of the European Society for Toxicology (in press).

Shah, H. C. and McLachlan, J. A.: The fate of diethylstilbestrol in the pregnant mouse. J. Pharmacol. Exp. Ther. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70080-03 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Study of Toxic Effects of Environmental Chemicals on Spermatogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. P. Lee	Pharmacologist	ETB NIEHS
OTHER:	R. L. Dixon	Chief	ETB NIEHS

COOPERATING UNITS (if any)

Hazleton Laboratories, Inc.

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Environmental Factors Affecting Germ Cell Development

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	2.0	1.6

SUMMARY OF WORK (200 words or less - underline keywords)

These studies seek to assess the effects of environmental agents on oogenesis and spermatogenesis. Environmental chemicals are tested, test systems validated and new approaches to toxicity testing proposed. To date, alkylating agents and selected trace metals have been studied. The DNA repair system in mouse germ cells appears useful as a predictor of toxicity. Biochemical and toxic effects of environmental agents on rodent spermatogenesis are also studied using velocity sedimentation cell separation and serial mating techniques. Enzyme development during spermatogenesis and "marker" enzymes have been defined which indicate normal cellular differentiation as well as toxic effects of chemicals. The nature of the blood-testis barrier and the penetration of environmental chemicals from blood to spermatogenesis cells have been reported. These studies contribute to an improved understanding of the reliability of laboratory animal toxicity tests for reproductive effects and the validity of their extrapolation to humans.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate whether the DNA repair system in mouse germ cells can be utilized as a predictive test system for detection of environmental mutagens; (2) to study biochemical and toxic effects of environmental agents on mouse spermatogenesis using both velocity sedimentation cell separation and serial mating techniques; (3) to define enzyme development during spermatogenesis and attempt to develop useful "marker" enzymes to indicate normal cellular differentiation and toxic effects of environmental agents on mouse spermatogenesis; (4) to characterize the interrelationship between hormone action and repression of testicular enzyme synthesis; and (5) to investigate the nature of the blood-testis barrier and the penetration of environmental chemicals to rat spermatogenic cells.

METHODS EMPLOYED: In vivo DNA repair was studied by labeling spermatogonial DNA with repeated i.p. administration of tritiated thymidine to 7 to 10 day old rats. Test mutagens were administered and seminiferous tubules were isolated. Spermatogenic cells were separated from tubules by tryptic digestion. Elution profiles defining DNA molecular size were obtained by akaline sucrose elution.

Dominant Lethality. Studies using male mice and rats were conducted using the following compounds: sodium tetraborate, methyl methane sulfonate, and aluminum.

Isolation of Spermatogenic Cells by Elutriation Centrifugation. Seminiferous tubules from adult mice were isolated and minced. Minced tubules were incubated with trypsin and tryptic action was stopped by addition of a trypsin inhibitor. Spermatogenic cells were washed and centrifuged three times after filtering through lens paper. One hundred to five hundred million cells were loaded in an elutriative rotor and 0.5% albumin containing Dulbeco-phosphate buffer was allowed to flow through the elutriative chamber at varying speeds to achiever counter current cell separation. Six spermatogenic cell types were separated in sufficient number to determine their "marker" enzymes.

Determination of Spermatogenic Cell Enzymes. Hyaluronidase (H), sorbitol dehydrogenase (SDH), lactic dehydrogenase-X (LDH-X), alpha glycerophosphate dehydrogenase (GPDH), malate dehydrogenase (MDH), and glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH) were assayed in both seminiferous tubules and isolated spermatogenic cells using standard methods. In order to study hormonal regulation of enzymes, specific activities were studied in relation to chronological age and the morphological development of the germ cells. Results were compared with spermatogenic enzyme development in hypophysectomized mice and hypophysectomized mice supplemented with androgens.

Preparation of Rat Testis for Blood-Testis Barrier. Efferent ducts of rat testes were ligated 18 hours before cannulating the rete-testis using microcapillary tubes. Rete-testis fluid was collected and cadmium was administered to study its permeability. Constant infusion of cadmium was used to establish

blood levels and to characterize the permeability of cadmium across the blood-testis barrier.

MAJOR FINDINGS:

DNA Repair in Spermatogenic Cells. These studies are being continued by Dr. Lee in Europe. No new findings are available. He is currently studying with Dr. Zbinden, Institute of Toxicology, Federal Institute of Technology and University of Zurich, Switzerland.

Dominant Lethality Studies. Boron, AF_2 and nitrous oxide had no mutagenic effect on either rats or mice. However, high levels of borax (500, 1000, and 2000 ppm per day as boron equivalents for 30 and 60 days) resulted in anti-spermatogenic effects in rats. The duration of infertility was dose dependent and correlated positively with increased plasma FSH levels. Both "marker" enzymes and morphological changes induced by boron indicated an initial effect on spermatids similar to hypophysectomy.

Investigations to assess the gonadotoxicity of aluminum are continuing. Groups of 30 adult male rats have been exposed to either zero level, 44.8, 447.6, or 4476 mg/liter of aluminum chloride ($AlCl_3 \cdot 6H_2O$) in drinking water. These dose levels extrapolate to 5, 50, and 500 mg/liter of aluminum and 0.005, 0.05, 0.5 mg/kg/day. Animals are exposed via drinking water for 90 days. Water consumption and body weight are monitored weekly. At 30, 60, and 90 days, groups of 7 animals are killed and clinical and histopathologic alterations evaluated. Following the 90 day exposure period, the reproductive capacity of groups of 10 male animals from each treatment group was assessed by serial mating. Corpora lutea, implantation sites, resorption sites, live fetuses, and dead fetuses were determined. Pregnancy rate and number of offspring per litter were also recorded. Serum gonadotropins (luteinizing hormone and follicle stimulating hormone) were monitored following the 30, 60, and 90 day exposure periods. No chemical-related abnormalities in any parameter have been apparent to date. The implantation efficiency (implantation sites/corpora lutea), incidence of resorption (resorption sites/implantation sites), and incidence of fetal viability (liver fetuses/implantation sites) were each undistinguishable from controls.

Spermatogenic Cell Enzymes. Postnatal development of testicular enzymes associated with germ cell differentiation in CD-1 mice was studied. Eight enzymes were selected: hyaluronidase (H), lactate dehydrogenase isoenzyme-X (LDH-X), dehydrogenases of sorbitol (SDH), α-glycerophosphate (GPDH), glucose-6-phosphate (GPDH), malate (MDH), glyceraldehyde-3-phosphate (G3PDH), and isocitrate (ICDH). Enzyme activities in testicular homogenates were determined by kinetic assays using spectrophotometry. Two types of enzyme developmental patterns were observed. One was represented by H, LDH-X, SDH, and GPDH; and the other, by G6PDH, MDH, G3PDH, and ICDH. The former was characterized by a change in enzyme activities from low in newborn to high in adult, while in the latter this development was reversed with the two complementary enzyme systems crossing each other at puberty.

The distribution of SDH and G6PDH in epididymis and vas deferens was also studied. Epididymis and vas deferens of adult mice were high in SDH and low in G6PDH, whereas the reverse was true in newborn mice. Histological observation indicated a close correlation between the sequential changes in enzyme activities and stages of spermatogenic cell differentiation.

Blood-Testis Barrier. Physiological studies have described the existence of a blood-testis barrier (BTB) surrounding the seminiferous tubules of the mammalian testis. These studies were initiated to better define the role of the BTB with regard to the penetration of exogenous chemicals to male germ cells. Permeability of nonelectrolytes of various molecular sizes, acidic compounds with varying partition coefficients and pK_a values, such as salicylic acid, barbiturates, and sulfonamides, across the BTB were studied. Permeability of nonelectrolytes was demonstrated to be dependent upon their molecular size, suggesting bulk flow through water-filled pores. On the other hand, permeability of acidic drugs with varying pK_a values depended upon their partition coefficients. Transport of these chemicals from blood to seminiferous tubules closely resembled their transport from blood to cerebrospinal fluid. It appears that the BTB is a complex multicellular system composed of membranes surrounding the seminiferous tubules and the several layers of spermatogenic cells organized within the tubules, which restrict the permeability to the male germ cells of many foreign compounds.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Studies of toxic effects of environmental agents on reproduction in the past centered largely on teratogenicity and dominant lethal studies. Few investigators have been concerned with the effects of chemicals on fertility and mechanisms of reproductive toxicity. Studies of spermatogenic enzymes associated with spermatogenic cell differentiation and aging will not only help to explain the mechanism of toxic effects, but will also be useful as biochemical "markers" to predict the type of spermatogenic cells that are damaged by toxic agents. Studies of the blood-testis barrier are important to the understanding of how various environmental agents enter the testis and exert toxic actions on germ cells. These types of biochemical and physiological studies are essential in assessing the potential hazards of environmental chemicals to male reproductive function as well as their mutagenic potential at the germ cell level.

PROPOSED COURSE: Future studies will continue to assess the effects of environmental agents on oogenesis and spermatogenesis. Longer term objectives include a much more expanded effort to identify environmental factors which account for male and female infertility. Close working relationships will be established with infertility clinics and scientists working in the area of epidemiology. More specifically, the following research activities are proposed: (1) identify marker enzymes which are specific for germ cell type to detect abnormalities in spermatogenesis and oogenesis; (2) investigate the effects of environmental agents on the regulation of differentiation and maturation of germ cells on a molecular level--correlate regulatory processes with morphological and ultrastructural abnormalities; (3) screen selected environmental agents for effects on germ cell development and function; and (4) evaluate epidemiological data

regarding chemically-induced effects on fertility and reproductive tract function.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70100-04 ETB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Homotypic and Heterotype Interactions in Chimaeric Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: L. A. Moustafa Senior Staff Fellow ETB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Toxicology Branch

SECTION

Developmental Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this project is to study chemical effects on differentiation as well as to investigate the biochemical and genetic mechanisms involved in early development and implantation. Areas of current interest include: (1) the determination of the role, fate, and the degree of cellular interaction of known transplanted single cell(s) in the development of host embryo; (2) the relationship of concentration and period of exposure to selected agents which induce embryopathy; and (3) the characterization of basic biochemical, physiologic, and genetic damage in vitro and in vivo.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: (1) To develop a system for determining the role and fate of known transplanted single cell(s) (at a definite stage of differentiation and/or treatment) in the development of host blastocysts (rabbits and mice); (2) to determine the type and degree of cellular interactions in the resultant chimaeras; and (3) to investigate the effects of selected treatment of preimplantation embryos and/or selected cells, which are to be transplanted, upon subsequent embryonic development.

METHODS EMPLOYED: Experiments involved four approaches: (1) chimaerism was induced by a micrurgical technique (Moustafa, L. A. and Brinster, R. L., J. Exptl. Zool., 181: 181, 1972) in which specific types and numbers of cells were isolated and transplanted into preimplanted mammalian embryos; (2) the complex homotypic and heterotypic (cell of like and unlike developmental history and properties, respectively) interactions in the resultant chimaeric young were investigated by observing the cell itself (genetic marker, e.g. pigmentation layer of the retina, albino, or pigmentation...etc.), the cell product (such as hair pigment or electrophoretic enzyme variants), and test breeding to determine whether the transplanted cell progeny can colonize the germ line and produce functional gametes; (3) determination of the concentration and period of exposure of selected agent(s) which induce embryopathy (e.g. monofunctional alkylating agent, or irradiation (MMS-induced lesion is assumed to be a single-brand break, while the UV-induced thymidine dimers); and (4) determination of the extent of DNA damage and repair in vitro and in vivo. Treatments were performed in vitro at various stages of preimplantation. In all procedures, micromanipulated embryos were cultured for developmental studies in vitro or until transferred into recipient females for the in vivo studies. Recipients were scheduled for Cesarian section on the last day of gestation. Fetal viability was recorded, gross congenital malformations noted, and live fetuses were placed with lactating foster mothers which had given birth within the previous 24 hours. Genetic-phenotypic determination of the animals were investigated at maturity.

MAJOR FINDINGS: Transplanted cell(s) can participate in normal morphogenesis and differentiation when restored to the environment of the preimplantation embryo. We have obtained unequivocal normal chimaeras following cell transplantation into intact rabbit blastocysts. Overt chimaerism was determined by inspection of the coat and eyes for the presence of pigmented melanocytes. Additional indicators of successful transplantation were: (1) disproportionate sex ratio favoring the male among the experimental young 66.5:33.5♂:♀ ($P<0.05$) and (2) incidence of sterility among resultant chimaeras was 12% and 33% among phenotypic females and males, respectively. Among chimaeras which produced young, overt signs of germ cell chimaerism were found in 29% and 30% of the litters of chimaeric females and males, respectively. These results (effects) seem to occur even in non-overt chimaeras that are phenotypically indistinguishable from the recipient strain. The possibility of "artifical hybridization" was indicated by the changes in body weight of resultant chimaeras after changing the origin of the transplanted cells.

The effects of alkylating agents (MMS) and ultraviolet irradiation on the developmental capacity of preimplantation embryos growing in vitro or after transfer in utero were also determined in mice. The effects were determined as a function of dose per unit time, function of time with defined dose stage of development at the time of administration with defined dose of the agents. Different UV radiation levels were required to produce equal effects on the survival of 2, 4, 8, morula, and blastocysts.

The relationships between embryonic survival after UV irradiation and alkylation of DNA by MMS were similar. In contrast, the response to a teratogen (e.g., thalidomide) differed markedly.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The analysis of genetic chimaeras has allowed inferences to be made about several otherwise elusive aspects of development including cell determination, division rates, lineage and deployment, and the sizes of pools of precursor cells destined to form particular organs and tissues. Of particular concern is whether a transplanted cell from an embryo with high resistance to an environmental agent would somehow transmit this desideratum to a susceptible recipient embryo; also, whether an auto-immune disease of an embryo can be inhibited by transplanting specific types of cells.

It has been suggested that UV mutagenesis in the eukaryote is an enzymatic process with mutations arising as a result of errors made during the repair of damaged DNA. Such repair has been called "error prone." Evidence for repair of damaged DNA in mammalian preimplantiation embryos has been detected in vitro and in utero; however, the survival rate was very low which may indicate an "error prone" repair?. If a chimaera will develop (carrier of "repaired-cell") and if such cell would colonize the germ line and produce functional gametes, it might be possible to produce animals from cells selected to carry various mutations. Animals which are models for genetically caused human diseases might also be developed.

PROPOSED COURSE: After obtaining a suitable number of chimaeric mammals, complete studies of host-transplanted cell interactions and animal phenotypes will be conducted. In addition, we will investigate the possibility of producing an interspecific chimaera, where antigenic differences between the two cell populations may be detected during all developmental stages. Isolated teratocarcinoma cells (treated or untreated with specific carcinogens) will also be utilized to produce chimaeras. Resultant chimaeras will be investigated cytologically, histochemically, and biochemically to study the chemical effects on teratocarcinoma cell differentiation as well as to investigate the biochemical and genetic mechanisms that control teratogenesis.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70120-05 ETB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Metabolism and Binding of Steroids and Xenobiotics in Developing Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. W. Lucier	Head, Develop. Tox. Group	ETB NIEHS
OTHER:	J. A. McLachlan	Research Associate	ETB NIEHS
	C. M. Schiller	Senior Staff Fellow	ETB NIEHS
	S. N. Nayfeh	Dir., Endocrine Labs	UNC

COOPERATING UNITS (if any)

Department of Biochemistry, UNC School of Medicine

LAB/BRANCH

Environmental Toxicology Branch

SECTION

Developmental Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range plan of this research project to study steroid metabolism and steroid binding in target tissues with emphasis on effects of hormonally-active xenobiotics. The studies will include the characterization of cytoplasmic and nuclear receptors in developing tissues of normal and abnormal animals. We also plan to study placental transfer and placental and fetal uptake and retention of hormonally-active xenobiotics. Recent emphasis has involved the perinatal development of steroid conjugation in rodents and the ontogeny of steroid cytoplasmic and nuclear receptors in target tissues in relation to gestational exposures to diethylstilbestrol and 2,3,7,8-tetrachlorodibenzo-p-dioxin.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: (1) To investigate possible enzyme multiplicity and enzyme specificity for glucuronyltransferase during perinatal development in different tissues, for example, determine whether there are different glucuronyltransferases responsible for the glucuronidation of steroids and exogenous chemicals; (2) to characterize conjugation pathways for hormonally-active xenobiotics; (3) to determine if and how environmental chemicals alter the normal developmental pattern of glucuronyltransferase and β-glucuronidase; (4) to characterize the biochemical indicators that are associated with the development of conjugative enzyme activity; (5) to study the role of steroid receptors in developmental and reproductive toxicity; (6) to study the role of steroid metabolism in developmental and reproductive toxicity; and (7) to study placental transfer and fetal uptake and retention of hormonally-active xenobiotics.

METHODS EMPLOYED: Tissue fractions from pregnant and non-pregnant animals were prepared using standard procedures. Rapid radiometric techniques were developed in this laboratory to measure conjugative enzyme activity in vitro, based on the partition of non-polar compounds into non-aqueous scintillation fluid. These methods are considerably more sensitive than colorimetric procedures and were used specifically to measure glucuronidation of steroids and non-steroids, β-glucuronidase, and N-acetyltransferase. The principle of these methods can be applied to the measurement of a wide variety of enzyme reactions.

Cytoplasmic receptors for ^{3}H-estradiol were measured in mouse vaginal and uterine tissue preparations by a microassay involving separation of free and bound steroid on DEAE-filters. Dissociation constants, quantification of receptors, and non-specific binding sites were determined on Scatchard plots. Nuclei were prepared and purified according to standard procedures for the purpose of determining nuclear uptake and binding of estradiol. The nature of nuclear-estradiol interactions was determined on sucrose gradients.

Steroid reductase and hydroxylase activities were determined using radioactive substrates. Estrone-estradiol interconversions were evaluated in mouse uterus cytosol and the respective steroids resolved on DEAE-cellulose columns.

^{109}Cadmium was injected i.v. to pregnant rats at the 12th, 16th, and 20th day of gestation and maternal liver, maternal blood, placental, and fetal cadmium levels measured 24 hours after treatment. The properties of binding proteins were studied according to M.W., amino acid content, and isoelectric focusing criteria.

MAJOR FINDINGS: Mouse vaginal cytoplasmic receptors for estradiol developed early in neonatal life reaching adult levels 10 days after birth, well before sexual maturity. In contrast, mouse uteri exhibited low levels of the cytoplasmic receptor prior to maturity; adult animals had three times the number of uterine receptors than were detectable 10 days after birth. Vaginal cytosol

receptors, at 21 days of age, exceeded the amount of uterine receptors whereas after 35 days uterine receptors predominated. There appeared to be no changes in receptor properties during development in either tissue as indicated by dissociation constants (approximately 10^{-10} M).

In animals exposed to DES (10μg/kg during days 10-16 of gestation), there were no significant changes in the numbers of uterine or vaginal cytoplasmic receptors or dissociation constants at any of the developmental stages tested. This dose schedule causes infertility in female offspring. In vivo uptake of β-estradiol by vaginal and uterine nuclei appeared to be decreased in adult animals exposed to DES prenatally. Gestational DES treatment did not affect β-estradiol uptake into the genital tract of male offspring. The equilibrium of estradiol-estrone interconversion appeared to shift towards estradiol in DES-exposed female offspring.

The perinatal development of steroid glucuronidation was investigated in mice and rats. Substrates used in these investigations were radiolabeled β-estradiol, testosterone, and DES. In rats, enzyme activity was first evident at the 18th day of gestation for all three substrates and activity reached adult levels by two weeks after birth. The fetal developmental pattern for glucuronyltransferase correlated well with levels of steroid glucuronide detected in fetuses at various developmental stages. On day 15 of gestation, approximately 5% of total radioactivity in fetuses represented glucuronides contrasted to 50% at day 21 of gestation. For DES, the nonconjugate fraction appeared to represent parent compound. Non-steroid glucuronidations (1-naphthol and p-nitrophenol) exhibited high activities in fetuses as evidenced by the perinatal development of non-steroid glucuronyltransferase, fetal metabolism, and tissue distribution studies. Non-steroid glucuronidations were induced by TCDD whereas steroid glucuronidations (including DES) were unaffected. We concluded that DES was conjugated as a steroid.

The placental transfer rates of cadmium increased with increasing dose and increasing gestational age (12-20 days) after cadmium injection. Placental sufficiency tests and histological evaluations indicated that increased placental transfer was not related to placental damage. Preliminary evidence suggests that effects on cadmium transfer is related to metallothionein inducibility characteristics.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Considerable research is in progress regarding the characterization of steroid metabolism and steroid receptors. However, relatively little attention is accorded the role that steroid metabolism and steroid binding play in developmental and reproductive toxicity. Our studies attempt to characterize receptors as "markers" for certain types of carcinogenesis and teratogenesis. Of particular interest is the ability of xenobiotics to alter neonatal "programming" of steroid metabolism and the role that such alterations might have in developmental toxicity.

Perinatal development studies of drug-metabolizing enzymes have in the past dealt primarily with oxidative systems. However, the conjugative systems, especially, glucuronidation, are in many cases more important in fetal detoxication and steroid regulation pathways than are the mixed function oxidases. The few developmental studies that were done on conjugative systems were incomplete; dealing with only one enzyme in one tissue at one age and often using unsuitable incubation conditions. Our studies will yield information concerning: (1) which organ or organs are most important in conjugation at different ages; (2) the type of fetal conjugate that is likely to be formed at particular times during the gestational peroid; (3) how the fetus independently detoxifies foreign compounds; (4) the role of conjugation pathways in the regulation of many endogenous compounds; (5) the mechanisms responsible for developmental onset of GT; and (6) the role that metabolism and chemical structure plays in the placental transfer of hormones and xenobiotics.

PROPOSED COURSE: We plan to concentrate our efforts for FY 77 on studying steroid metabolism and binding in target tissues with emphasis on effects of hormonally-active xenobiotics. The studies will include the characterization of cytoplasmic and nuclear receptors in developing tissues of normal and abnormal animals. Dr. Lamartiniere will begin work at the NIEHS in May 1976, and he will study the reversible and irreversible neonatal imprinting of steroid metabolizing enzymes. These investigations will attempt to elucidate developmental enzyme patterns and to identify which systems are sensitive to chemical insult. The long-term goal of this research will be to characterize biochemical indicators of developmental toxicity that have clinical value.

During FY 77, we will also investigate placental transfer and fetal retention of hormonally-active xenobiotics such as the PCBs, emphasizing binding to hormone-receptor molecules in the fetus.

PUBLICATIONS

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70130-01 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Metabolic Energy Producing Processes in Developing Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. M. Schiller Senior Staff Fellow ETB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Developmental Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	2.5	0.6

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the perinatal development of the functioning small intestine in normal and environmentally, nutritionally, and hormonally stressed animals. The topics of present interest which relate to the development of the metabolic energy producing processes are: (1) the establishment of the mitochondrial permeability characteristics and substrate shuttles for NADH in the intestinal mucosa of hamsters; (2) the developmental patterns of the shuttle enzymes; (3) the relative contribution of the segments and mucosa and serosa to these activities; (4) the development of pyruvate dehydrogenase in relation to the redox state of the intestine; and (5) the establishment of isolation techniques and culture conditions for crypt and tip cells from the mucosal wall.

PROJECT DESCRIPTION

OBJECTIVES: (1) To establish the presence of the NADH reducing equivalent shuttles in adult small intestinal mucosal mitochondria; (2) to evaluate the developmental patterns for the enzymes of the substrate shuttles in the small intestine; (3) to determine the contribution of the various segments along the small intestine as well as that of the mucosa and serosa to the total shuttle enzymic activities in the small intestine of the young and adult; (4) to monitor techniques for isolating epithelial cells both tip and crypt from the serosal wall of the young and adult; (5) to establish growth and maintenance conditions for the epithelial cells in primary monolayer culture; (6) to relate the total (activated) and basal levels of pyruvate dehydrogenase in the developing small intestine to the redox state of the organ in the perinatal period; (7) to consider possible exogenous compounds to utilize as an effector in these systems; (8) to study transport of exogenous substances with isolated intestinal epithelial cells from animals treated with pesticides and heavy metals; (9) to utilize the isolated epithelial cells as an *in vitro* test system for ingested substances; (10) to investigate transplacental carcinogens that effect the intestine; (11) to utilize the mitochondrial and enzyme techniques to study the effects of chronic Kepone and related compounds exposure to animals; and (12) to collaborate on a study dealing with the chemically induced alterations in neonatal programming of steroid metabolism and binding.

METHODS EMPLOYED: The preparation of subcellular fractions and mucosal scrapings and the measurement of enzymes, metabolites, and substances were performed by adaptation of standard procedures. It was necessary to modify the procedure for the preparation of the small intestinal mitochondria for the permeability and reconstructed shuttles studies. Recording spectrophotometric enzyme assays were utilized. A linked-enzyme assay requiring pyruvate kinase and lactate dehydrogenase was developed to measure Mg^{+2} ATPase activity in the mitochondrial preparation. All substrates and cofactors required were available commercially. The pyruvate dehydrogenase levels were measured by the liberation of ^{14}C carbon dioxide from sodium pyruvate (1-^{14}C). All assays were standardized under optimal conditions for linearity with protein concentration and with time. Membrane permeability was analyzed by oxygen consumption with various substrates, e.g., NAD(P)H, succinate, pyruvate/malate, glutamate/malate, and α-glycerol phosphate, and by decreased absorbance of NADH in reconstructed shuttles with an oxygen electrode and a DW-2 Aminco spectrophotometer, respectively.

Eight different aged animals were used in the developmental studies to detect changes in the specific activities and subcellular distribution (cell debris, mitochondria, and cytosol) of the shuttle enzymes (aspartate aminotransferase, malate dehydrogenase, NAD$^+$-α-glycerol phosphate dehydrogenase, and Fp-α-glycerol phosphate dehydrogenase) and lactate dehydrogenase (a marker for anaerobiosis and the cytosol) and citrate synthase (a marker for aerobiosis and the mitochondria). At the earlier stages, the whole intestines of two litters were pooled, while at the later stages individual small intestines were used. At the adult stage, male and female small intestinal tissues were also compared.

The shuttle enzymes were also measured in the total small intestinal mucosa and in the segments along the length of the small intestine in both the mucosa and serosa in the adult male and 10-day old hamsters.

The contribution of the basal level to the total (activated) pyruvate dehydrogenase activity during eight stages of development was related to the redox state of the organ. The redox state of the cytosol and of the mitochondria was determined by measuring the lactate to pyruvate and acetoacetate to β-hydroxybutyrate ratios, respectively.

Epithelial cells isolated from the small intestine of adult male and 10-day old hamsters by eversion and agitation followed by insufflation and agitation were monitored with time for the release of protein, DNA synthetic activity, alkaline phosphatase, acid phosphatase, β-glucuronyltransferase, and β-glucuronidase. These preparations were monitored for characteristic morphological structures. Initial studies were performed to maintain intestinal epithelial cells in primary culture utilizing techniques and media currently used for intestinal organ culture and other epithelial cell cultures.

MAJOR FINDINGS: Mitochondria from hamster small intestinal mucosa were prepared, exhibiting good respiratory control indices. They would not oxidize NADH or NADPH. The presence of N,N,N',N'-tetramethyl-p-phenylenediamine permitted the transport of reducing equivalents from NADH. These mitochondria were found to oxidize both L-glycerol 3-phosphate and L-glutamate plus L-malate at significant rates. Each of the necessary enzymes was demonstrated in the appropriate subcellular fractions as required for the function of the reducing equivalent shuttle systems. The small intestinal mucosal mitochondria exhibited adequate rates of cytosolic NADH oxidation in both of the reconstructed shuttle systems.

Changes in specific activity levels of lactate dehydrogenase, citrate synthase, NAD^+-linked L-glycerol 3-phosphate dehydrogenase, Fp-linked L-glycerol 3-phosphate dehydrogenase, L-aspartate aminotransferase, and L-malate dehydrogenase in the whole intestine and its subcellular fractions were studied during the development of the hamster from three days before birth to the adult stage. A diversity of developmental patterns was observed among the various enzymes. However, each of the enzymes of the substrate cycles for the transport of reducing equivalents from cytosolic reduced nicotinamide adenine dinucleotide to the mitochondria showed marked increases at the 7-day postnatal period. Both the level of activity and the appropriate subcellular locations of activity of each of the substrate cycles were present for the function of the L-glycerol 3-phosphate and L-malate/L-aspartate substrate cycles through the stages of development studied. There was no significant difference based on sex in the levels ($p<.01$) of these enzymes in the adult animals.

A comparison of the specific activities of the shuttle enzymes in the small intestinal mucosa of the adult male and 10-day old hamster indicated that only the two α-glycerol phosphate dehydrogenases were significantly higher in the adult mucosa ($p=.01$). At both stages, the total activities of the enzymes

were higher in the three mucosal segments than in the three serosal segments. This difference was more pronounced in the adult segments. With the exception of the NAD^+-α-glycerol phosphate dehydrogenase, the enzymes of the mucosal segments were observed to have higher or similar specific activities than those of the serosal segments. In the 10-day old hamster, there was a marked increase in the specific activity of the NAD^+-α-glycerol phosphate dehydrogenase in the serosal segments.

When the developmental pattern of pyruvate dehydrogenase was observed for eight stages, a marked increase in the basal to total ratio activity was seen at the 1-day postnatal stage, but the total (activated) pyruvate dehydrogenase level increased at 5-day old stage.

The developmental patterns for the enzymes involved in metabolic energy production observed indicate that: (1) the substrate shuttles may play an important function in NADH transport at the stages studied and (2) the mucosa is not as well developed in the 10-day old animals in terms of total activity but is similar to the adult in specific activity. The proportion of the total pyruvate dehydrogenase activity which is active in the 1-day old hamster may indicate a site of regulation to permit greater oxidation of substrate as required to meet the greater demands during the perinatal period. This change in ratio precedes a change in the total activity at 5 days.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Perinatal development studies of the small intestine have in the past dealt primarily with hydrolytic enzymes, the brush border, and the absorption of specific nutrients. However, energy metabolism, especially the development of the metabolic energy producing processes which form the basis for the active transport for monosaccharides and amino acids, has been overlooked. The few developmental studies that were performed in energy related areas dealt with the onset of active transport during the development of the chick and the appearance of mitochondria in the columnar epithelial cells. These studies have provided information concerning: (1) the permeability characteristics of the mucosal mitochondria and the functioning of the aspartate/malate and α-glycerol phosphate shuttles in the adult mucosa; (2) the developmental pattern of the shuttle enzymes and marker enzymes in the whole intestine; (3) the enzymic differences between adult male and female and 10-day old animals; (4) the enzymic differences in the segments of the intestine in the mucosa and serosa of adult male and 10-day old animals; (5) the developmental pattern of pyruvate dehydrogenase as it relates to the redox state of the intestine; (6) the isolation and culture of cyrpt and tip cells from adult and 10-day old hamsters.

A correlation between the change in redox state during the perinatal period and the proportion of the total pyruvate dehydrogenase activity which is active at birth will provide insight to understanding how this tissue converts from anaerobiosis to aerobiosis. Observations from these studies provide new information as to how there can be high aerobic glycolytic rates in this tissue, i.e., a lack of a Pasteur effect.

PROPOSED COURSE: We plan to concentrate our efforts for FY 77 on studying transport of exogenous substances with isolated intestinal epithelial cells from animals treated with pesticides and heavy metals. We will look at the induction of β-glycuronyltransferase and β-glucuronidase in the crypt and tip cells of normal and induced animals. The primary epithelial cell culture system will be used to study the effects of xenobiotics on epithelial cell functions and growth. We will utilize the isolated cell and cell culture systems to study the development of active transport of nutrients in mammals. We will consider testing compounds for transplacental toxic effects on the intestinal development. The mitochondrial and enzymic techniques we have developed will be used to study the effects of chronic Kepone and related compounds exposed to animals. Collaboration with my workgroup will focus on the chemically induced alterations in neonatal programming of steroid metabolism and binding.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70140-04 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Factors Affecting Hepatic Heme Biosynthesis and Its Utilization During Mammalian Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. S. Woods Head, Biochem. Tox. Group ETB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Biochemical Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are designed to investigate the role of heme in the regulation of the biosynthesis, assembly, and functional activation of hepatic hemoproteins during mammalian development. A functional association between heme synthesis and the biosynthesis of mitochondrial hemoproteins is suggested by the accelerated rate of both processes in fetal liver and the rapid decline in the rate of mitochondrial protein synthesis observed when heme synthesis is inhibited. Heme appears to stimulate new apocytochrome oxidase synthesis in the fetus and mediates the assembly of apoprotein and heme into a functional cytochrome. Prenatal exposure to agents which inhibit hepatic heme biosynthesis results in delayed or inhibited postnatal development of both mitochondrial and microsomal hemoprotein function. Thus, heme may be rate-limiting in the synthesis of both mitochondrial and microsomal hemoproteins during mammalian development.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this project are: (1) to determine a functional correlation between hepatic heme biosynthesis and the biogenesis of hepatic hemoproteins in fetal mammalian liver; (2) to evaluate the nature of the specific role of heme in the synthesis and assembly of hepatic cytochromes, specifically cytochrome oxidase and cytochrome P-450; (3) to investigate the relationship between hemoprotein synthesis and the biogenesis of mitochondria in fetal liver; (4) to determine the mechanisms by which agents that specifically interfere with hepatic heme synthesis might alter the functional development of metabolic and/or drug-metabolizing enzymes in fetal liver; and (5) to provide a means for predicting the potential transplacental hematotoxicity of environmental agents in humans.

METHODS EMPLOYED: All studies will be conducted using hepatic tissues of adult and fetal rats at 18 days of gestation. In in vivo studies, drugs will be administered to the fetus by IP or IV injection of the mother. Studies conducted in vitro will utilize the pooled tissue homogenates of at least 3 litters or adult animals for each experimental point. Data will be analyzed using commonly accepted statistical methods.

Various approaches will be utilized to assess the role of heme in the regulation of the synthesis and functional activation of hepatic cytochromes in fetal liver. These will include: (1) assessment of the role of heme in preventing or reversing the decline in both cytochrome oxidase and the rate of synthesis of mitochondrial protein components following inhibition of these synthesis in vivo; (2) studies on the effects of heme on the turnover of hepatic cytochromes following specific inhibition of ribosomal or mitochondrial protein synthesis in vivo; and (3) studies to determine the role of heme in the formation of mitochondrial and microsomal holocytochromes from the apoprotein following inhibition of heme synthesis in vivo and in vitro. Rates of protein and heme biosynthesis will be assessed using pulse-labeling with C-14-leucine or H-3-aminolevulinic acid, respectively. Time course studies will be performed to assess the sequential relationship between heme and apoprotein synthesis following changes in heme levels in vivo. In the assessment of transplacental hematotoxicity, biochemical, and morphological studies will be performed to determine the effects of prenatal exposure to various hematotoxic agents on the maturation of hepatic hemoprotein function.

MAJOR FINDINGS: Studies conducted thus far indicate that heme plays an important, but probably indirect, role in regulating the overall synthesis of mitochondrial proteins in fetal mammalian liver. This action seems to be mediated more directly through stimulation of the synthesis of cytochrome oxidase and, perhaps, other mitochondrial hemoproteins which form an integral portion of the mitochondrial structure. The direct action of heme appears to be mediated through stimulation of the synthesis of the apoprotein and facilitation of the assembly of the apoprotein and heme moities into a functional cytochrome. Prenatal exposure to agents which selectively interfere with hepatic heme biosynthesis in the fetus produce impaired or delayed maturation of both mitochondrial

and microsomal hemoprotein function during postnatal development. Thus, transplacental hematotoxicity during late gestation may be manifested as enhanced susceptibility to the toxic effects of drugs and other agents during the immediate postnatal period.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Knowledge of the mechanisms by which environmental agents selectively exert toxic effects on postnatal growth and function is essential to the development of a biochemical basis for both understanding and preventing transplacental organ toxicity. The proposed studies are designed to develop such knowledge in the area of hematopoietic function through investigation of the mechanisms of regulation of heme biosynthetic capability and of hemoprotein function in mammalian liver during development, and through elucidation of the effects of prenatal exposure to hematotoxic agents on the postnatal development of specific regulatory hemoproteins. It is anticipated that these studies will contribute to an enhanced understanding of the problem of transplacental hematotoxicity and promote the more efficacious identification of potentially harmful hematotoxic environmental substances.

PROPOSED COURSE: Studies concerning the role of heme in the regulation and functional activation of hemoproteins in fetal mammalian liver will continue. Immediate emphasis will be placed on an investigation of the role of heme in the synthesis of cytochrome oxidase and the correlation of cytochrome oxidase synthesis with the biogenesis of mitochondrial proteins in general. Future studies will examine the role of heme in the regulation of extramitochondrial cytochromes and other regulatory hemoproteins in mammalian tissues at different stages of development.

PUBLICATIONS

Woods, J. S.: Studies on the Rate-Limiting Role of δ-Aminolevulinic Acid Synthetase in Heme Biosythesis in Fetal Rat Liver. In Karger, S. (Ed.): Porphyrins in Human Disease. Basel, 1976 (in press).

Woods, J. S.: Developmental aspects of hepatic heme biosynthetic capability and hematotoxicity. Biochem. Pharmacol. 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70200-03 ETB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Morphologic and Biochemical Effects of Toxic Metals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. A. Fowler	Senior Staff Fellow	ETB NIEHS
OTHER:	R. A. Goyer	Chairman, Dept. of Pathology	Univ. of W. Ontario
	K. R. Mahaffey	Asst. to Director	FDA
	J. S. Woods	Head, Biochem. Tox. Group	ETB NIEHS
	G. W. Lucier	Head, Devel. Tox. Group	ETB NIEHS
	G. E. R. Hook	Senior Staff Fellow	PB NIEHS
	M. R. Krigman	Professor of Pathology	UNC
	P. Mushak	Asst. Prof. of Pathology	UNC
	G. F. Nordberg	Associate Professor	Karolinska Inst.

COOPERATING UNITS (if any)

Department of Pathology, UNC
FDA, Washington, D. C.
Pharmacology Branch, NIEHS
Department of Environmental Hygiene, Karolinska Institute

LAB/BRANCH

Environmental Toxicology Branch

SECTION

Biochemical Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

When animals are chronically exposed to different trace metals, each metal produces an enzymatic response profile which specifically characterizes exposure to that metal. It is the objective of these studies to assess and characterize response profiles resulting from exposure to various environmentally important toxic metals and to apply knowledge of their response profiles to the design of tests (such as the ALA test currently available for the detection of lead) for use among human populations in detecting pre-clinical, i.e., pre-toxic, exposure to a given metal. Specific metals and areas of interest include the biochemical effects of methyl mercury, arsenic, lead, and lead plus fluoride on mitochondrial structure and function. Enzyme activities associated with mitochondrial membranes have been found to show early and pronounced changes in mitochondria of rats and mice following chronic exposure to methyl mercury and arsenic. Further development of these biochemical response profiles should permit development of metal-specific biochemical testing procedures which accurately assess a biological response to exposure from these agents.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: (1) To define and correlate ultrastructural and biochemical responses in vivo which characterize exposure to toxic trace elements following chronic exposure and (2) to develop early, specific, sensitive, biochemical testing procedures that may be used to evaluate human population exposed in utero or as adults to environmentally important toxic elements.

METHODS EMPLOYED: Quantitative transmission electron microscopy; x-ray microanalysis; biochemical assays of mitochondrial respiration; and microsomal, cytosol, and mitochondrial enzymes.

MAJOR FINDINGS:

(1) Arsenic. Combined ultrastructural and biochemical studies on liver mitochondria from rats and mice exposed to 0, 20, 40, or 85 ppm arsenate (As^{+5}) for 6 weeks produces in situ mitochondrial swelling correlated with decreased state 3 respiration and respiratory control ratios for pyruvate/malate but not succinate-mediated respiration. These findings are associated with alterations in mitochondrial membrane enzymes but not marker enzymes found in the cytosol, microsomes, or mitochondrial matrix. Interspecies comparisons show a consistent response profile characteristic of As^{+5} exposure.

(2) Lead-Fluoride. Rats given access to a semi-purified diet containing 0.1% lead and 50 ppm sodium fluoride showed decreased weight gains, increased femur lead values, but decreased urinary Δ-ALA concentrations in comparison with animals given a diet containing lead alone. Renal proximal tubule cells of animals given the lead-fluoride diet show intranuclear inclusion bodies but less apparent mitochondrial swelling than is observed in animals given a diet containing lead alone.

(3) Methyl Mercury. Rats and mice were given access to drinking water containing 0, 3, 5, or 10 ppm mercury as methyl mercury hydroxide for 6 weeks. Ultrastructural and biochemical studies of livers and kidneys from these animals disclose that renal mitochondria are more adversely affected than are hepatic mitochondria and that mice show a more pronounced and uniform response to this regimen than do rats. State 3 respiration and respiratory control ratios are decreased in mitochondria of both species. Activities of enzymes located on the mitochondrial outer membrane increased, while those of inner membrane decreased. These patterns are specifically characteristic of chronic exposure to methyl mercury.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies attempt to characterize and delineate the subcellular mechanisms of trace metal toxicity following chronic exposure by both ultrastructural and biochemical techniques. Once sufficient knowledge in this area is obtained, it may be applied to the development of metal-specific biochemical testing procedures which will accurately reflect a preclinical biological response to toxic trace metal exposure in human populations.

PROPOSED COURSE: Further studies on the subcellular mechanisms of trace metal toxicity are planned for arsenic and chromium. A contract for the screening of selected biochemical and physiological parameters following chronic oral or inhalation exposure to these elements can be initiated. Based on the result of these studies, tests of potential clinical utility will be devised to detect subtoxic exposure of human population to selected trace metals.

PUBLICATIONS

Fowler, B. A., Brown, H. W., Lucier, G. W., and Krigman, M. R.: The effects of chronic oral methyl mercury exposure on the lysosome system of rat kidney. Morphometric and biochemical studies. Lab. Invest. 32: 313-322, 1975.

Fowler, B. A.: Heavy metals in the environment II: An overview. Environ. Health Persp. 10: 259-260, 1975.

Fowler, B. A., Lucier, G. W., and Mushak, P.: Phenobarbital protection against methyl mercury nephrotoxicity. Proc. Soc. Exp. Biol. Med. 149: 75-79, 1975.

Fowler, B. A. and Goyer, R. A.: Bismuth localization within nuclear inclusions by X-ray microanalysis: Effects of accelerating voltage. J. Histochem. Cytochem. 23: 722-726, 1975.

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Fowler, B. A., Jones, H. S., Brown, H. W., and Haseman, J. K.: The morphologic effects of chronic cadmium administration on the renal blood vasculature of rats given a low and normal calcium diet. Toxicol. Appl. Pharmacol. 34: 233-252, 1975.

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Fowler, B. A., Fay, R. C., Walter, R. L., Willis, R. D., and Gutknecht, W. F.: Levels of toxic metals in marine organisms collected from southern California coastal waters. Environ. Health Persp. 12: 71-77, 1975.

Brown, M. M., Rhyne, B. C., Goyer, R. A., and Fowler, B. A.: The intracellular effects of chronic arsenic administration on renal proximal tubule cells. J. Toxicol. Env. Health 1: 507-516, 1976.

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Fowler, B. A.: The Toxicology of Environmental Arsenic. In Mehlman, M. and Goyer, R. A. (Eds.): Advances in Modern Toxicology III. The Trace Metals. Horizon Publishing Co. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70220-03 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Neurobehavioral Toxicity of Developmental Exposure to Lead

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. A. Kimmel	Senior Staff Fellow	ETB NIEHS
OTHER:	C. Shreffler	Staff Fellow	ETB NIEHS
	T. Damstra	Biochemist	OP NIEHS
	L. D. Grant	Assistant Professor	UNC

COOPERATING UNITS (if any)

University of North Carolina
Office of Program, NIEHS

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Behavioral Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of lead on the behavior and development of offspring exposed chronically before and after birth is being studied. A broad battery of observations of physical developmental milestones, reflex ontogeny, and behavioral functions is being employed to determine the full range of neurotoxic potential of lead. These observations are being related to the teratogenic activity and body burden of lead as estimated from levels found in blood, brain, and bone at various times throughout the exposure period and to neurochemical parameters which indicate changes in developing brain chemistry. The value of this multidisciplinary approach will be evaluated in terms of its usefulness for routine evaluation of compounds with unknown neurotoxic potential.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The primary objectives of this study are to investigate the effects of environmental agents (using lead as a prototype compound) on several facets of behavior of offspring exposed during development and to relate behavioral changes to morphological and chemical observations.

METHODS EMPLOYED: Female rats obtained at weaning are being exposed to the following doses of lead (as lead acetate) in deionized drinking water: 0, 0.5, 5, 50, and 250 ppm. Exposure continues for 6-7 weeks at which time animals are mated; then exposure continues through pregnancy, lactation, and subsequently to the offspring following weaning. At 21 days of gestation some animals are sacrificed and a routine teratology exam done. Other animals are sacrificed at various times throughout the exposure and blood, brain, and bone collected for analysis of lead by atomic absorption spectroscopy. Another group of animals is allowed to litter, and offspring are examined for physical milestones (e.g., eye opening, pinna detachment), reflex ontogeny (e.g., righting, visual placing), and later for locomotor coordination, activity, learning acquisition of several responses, and sexual behavior. A final group of animals is sacrificed at various times and levels of acetyl- and butylcholinesterase (indicating neuron and glial proliferation, respectively) are determined.

MAJOR FINDINGS: Concentrations of 0.5 and 5 ppm of lead have no effect on maternal growth or weight gain during pregnancy. At 50 and 250 ppm, lead significantly retards growth, delays vaginal opening, and interferes with weight gain during pregnancy. However, no reproductive, teratogenic, or embryolethal effects are seen at any dose level. Reflex development and body weights are also significantly affected in offspring exposed *in utero* to 50 and 250 ppm, but not at doses where there is no maternal toxicity. However, all behavioral tests are not yet completed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is an immediate need for the study and evaluation of toxicant effects on behavior and the assessment of such effects as early indicators of toxicity. These studies relate directly to the tremendous problems of congenital mental retardation and abnormal behavior in children for which there is, in most cases, no known etiology.

PROPOSED COURSE: The evaluation of the results of this study will be used to determine appropriate methods for screening of environmental agents for neurobehavioral toxicity. In addition, areas will be identified which require development of new and better methods for assessing behavior.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 70240-03 ETB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Study of the Molecular Mechanisms of Chemical Toxicity in Mammalian Cells Cultured In Vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. W. Spalding Research Biologist ETB NIEHS
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Environmental Toxicology Branch

SECTION

Biochemical Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The toxicity of chemical agents which may be hazardous to human health and to other species is being studied in mammalian cells cultured in vitro to gain an insight into the molecular mechanisms of toxicity. The toxicity of selected organochlorine agents has been demonstrated. The most striking effect of these agents on cell metabolism is concerned with phospholipid metabolism. In the mouse L5178Y lymphoma cell line, DDT, DDT analogs, and 4-chlorobiphenyl stimulate the rate of de novo synthesis of phosphatidycholine. There is a positive correlation between the relative toxicity of organochlorine agents and their ability to stimulate phosphatidylcholine (PC) synthesis. The effect of organochlorine agents on phospholipid metabolism varies with the cell line studied. Phosphatidylcholine in the mouse Balb C/3T3 fibroblast cell line was the least effected, while stimulation of the rate of PC synthesis was highest in human embryonic (WI-38) lung fibroblasts. The effect of organochlorine agents on PC synthesis in WI-38 cells is similar to that elicited by the tumor promotor, tetradecanoylphorbol acetate.

PROJECT DESCRIPTION

OBJECTIVES: To study the mechanism of chemical toxicity of environmental agents in mammalian cells cultured in vitro in order to gain insight into how these agents may effect human health.

METHODS EMPLOYED: The effect of organochlorine agents on macromolecular biosynthesis in mouse L5178Y lymphoma cells has been investigated by employing appropriate radiolabeled precursors. Since organochlorine agents have been demonstrated to have a specific effect on phospholipid metabolism, emphasis has been placed on methods of phospholipid isolation and the separation and recovery of the different phospholipid species. Subsequently, the effect of organochlorine agents on phospholipid metabolism is being compared in three different mammalian cell lines. The relative distribution of phospholipid precursors, e.g., $^{32}PO_4$, ^{14}C-methycholine, and ^{3}H-glycerol, into the major phospholipid components of these cells is being investigated. The relative activities of the enzymes comprising the de novo biosynthetic pathway of phosphatidylcholine are to be compared in treated and non-treated cells.

MAJOR FINDINGS:

(1) Since no net increase in total phospholipid or phosphatidylcholine (PC) can be detected in treated mouse L5178Y cells, the stimulation of ^{14}C-choline incorporation into PC by organochlorine agents appears to reflect an increase in the rate of PC turnover.

(2) 4-chlorobiphenyl selectively stimulates the incorporation of ^{3}H-glycerol into the phosphatidylcholine (PC) fraction of mouse L5178Y cells. Both 4-chlorobiphenyl and the tumor promotor, tetradecanoylphorbol acetate preferentially stimulate the incorporation of inorganic $^{32}PO_4$ into the PC component of the phospholipids isolated from treated WI-38 cells. These findings support the hypothesis that these agents are selectively stimulating the de novo synthesis of phosphatidylcholine.

(3) The commercial Aroclor mixtures, 1221, 1242, 1248, 1254, and 1016, as well as DDT and several DDT analogs, all stimulate phosphatidylcholine metabolism in human lung (WI-38) fibroblasts.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

(1) The mechanism of organochlorine toxicity is not known. It is reasonable to assume that the known lipophilic character of these agents is directly related to their mode of action. The demonstrated effect of these agents on phospholipid metabolism may well be associated with their mechanisms of toxicity.

(2) The physiological significance of phospholipid turnover is not understood. However, the stimulation of phosphatidylcholine turnover by toxic agents is likely to represent an initial injury response. The study of organochlorine toxicity in this model system and in other cells or tissues as well can lead

to an understanding of (1) how cells respond to injury by lipophilic compounds and (2) the nature of the physiological role of specific phospholipid species.

(3) This course of research has direct application to the study of how environmental agents may effect the regulation of dipalmitoyl phosphatidylcholine synthesis by the alveolar type II cell in the lung. Dipalmitoyl-phosphatidylcholine is the major phospholipid component of the surfactant lipoprotein lining the alveolar surface of the lung.

PROPOSED COURSE:

(1) The de novo synthetic pathway of phosphatidylcholine (PC) biosynthesis is comprised of three different enzymes: choline kinase, phosphorylcholine-cytidyl transferase, and phosphorylcholine-glyceride transferase. The activity of these enzymes in cell-free preparations can be measured by using the appropriate labeled substrates. The relative activities of these enzymes should be compared in untreated and treated cells.

(2) It should be determined whether or not organochlorine agents stimulate a calcium-dependent direct base exchange of ^{14}C-choline with pre-formed PC. This reaction is also studied in cell-free preparations.

(3) Two chemical agents, 4-nitrobiphenyl and 4-aminobiphenyl, are considered to be carcinogenic in man. These two agents are closely related structurally to 4-chlorobiphenyl. The effect of these two carcinogens on phospholipid metabolism should be compared in the model cell systems described above.

(4) It should be determined whether the stimulation of phosphatidylcholine turnover in response to the interaction of organochlorine agents is related to changes in intracellular cyclic-nucleotide levels.

(5) The effect of organochlorine agents, e.g., PCBs, and the tumor promotor tetradecanoylphorbol acetate, on the regulation of phosphatidylcholine biosynthesis in the Alveolar Type II cells of the lung should be investigated. These studies may be carried out in vitro in an isolated Aveolar Type II cell line, in primary cell culture preparations from lung tissue and compared with studies in vivo.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70280-03 ETB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Plasma Membranal Enzymes and Regulation of Mammalian Embryo Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. E. Wilson	Research Chemist	ETB NIEHS
OTHER:	J. R. Hass	Staff Fellow	EBCB NIEHS
	E. Soares	Senior Staff Fellow	EMB NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch
Environmental Mutagenesis Branch

LAB/BRANCH
Environmental Toxicology Branch

SECTION
Biochemical Toxicology Group

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.1	1.3

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this investigation is to attempt to ascertain the nature of developmental regulatory systems whose modification during embryogenesis may result in altered neural tube development (e.g., anencephaly, exencephaly, or spina bifida). The studies focus on: (1) the problems associated with staging teratogenic responsiveness in offspring of the rat or mouse; (2) characterization of the behavior of those plasma membranal enzymes (e.g., prostaglandin synthetase, $(Na^+ + K^+)$ATPase, and adenyl cyclase) which are likely to play a significant role in determining responsiveness of embryonic cells to teratogenic stresses, namely, those due to deficiencies in essential nutrients, to drugs and to environmental agents.

PROJECT DESCRIPTION

OBJECTIVES: (1) In order to anticipate the consequences of various types of maternal environmental exposure on mammalian embryo development, it will be necessary to obtain further insight into those biochemical systems and/or metabolic pathways which, when modified by a suitable perturbant, are causally related to teratogenic responsiveness. (2) To ascertain the extent to which modulation of plasma membranal structure and/or function might correlate with fetal toxic or teratogenic responsiveness. (3) To characterize the properties and behavior of plasma membranal regulatory enzymes which occur in endometrial, placental, and/or embryo-fetal tissues.

METHODS EMPLOYED: Feeding studies require the use of defined diets and occasional diet modification (addition of antimetabolites, drugs, etc.). Also required are facilities for mating and for anatomical (teratology) examination. Behavioral studies of embryo-fetal, uterine, and placental prostaglandin synthetases are performed using analytical techniques which can permit chemical identification of the prostaglandins.

MAJOR FINDINGS: The incidence of abnormal fetal development was not detectably different in offspring of essential fatty acid-deficient animals from that previously noted among normal rats which received a pantothenate-deficient diet during pregnancy. On the other hand, consumption of a diet devoid of non-vitamin lipid during pregnancy resulted in an increase in the incidence of resorptions hematomas and fetal deaths in certain litters.

Analysis of results accumulated from anatomical (teratology) examinations indicated that two (possibly, three) general types of responsiveness occur in rat embryo-fetal development as the consequence of maternal consumption of a diet deficient in pantothenate ($\pm$ ω-methyl pantothenate) prior to and/or during pregnancy. Litters from some rats comprised only normal-appearing offspring; other litters comprised one or more abnormally developed offspring, while still others were comprised only of resorption sites. These variations in responsiveness probably reflected one or more genetic peculiarities resulting in one or more pantothenate-dependent metabolic pathways which could be modulated by diet.

Aspirin in the diet at concentrations which have little effect in rats consuming a nutritionally adequate diet, markedly enhances abnormal fetal development in litters from rats fed a diet deficient in pantothenate. However, about 50% of the rats receiving the pantothenate deficient diet appeared to be about as resistant to aspirin as rats receiving a normal diet.

Teratogenicity of trypan blue resembled that resulting from the administration of a pantothenate deficient diet in that abnormal fetal rat development usually occurred in only a few individuals in litters from susceptible females.

Variations in prostaglandin (PG) synthetase activities of microsomes from rat uterine, placental, and embryonic tissues were measured at different stages of pregnancy. Uterine PG synthetase activity appeared to be relatively constant until term, then it increased several fold. Conceptus tissue at pregnancy days 11 and/or 12 had relatively high levels of PG synthetase; late placental tissue appeared to have little PG synthetase activity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Alterations in rat embryonic neural tube development which results from a mild teratogenic perturbation such as maternal consumption of a pantothenate deficient (PD) diet or of a PD diet supplemented with aspirin only during pregnancy appeared to be genetically determined. Only a better understanding of the biochemical basis(es) for abnormal embryonic development might permit anticipation of and/or protection against agents responsible for such development.

Our efforts have provided an indication of the variability of prostaglandin synthesis by tissue from the conceptus (presumably from the embryo) and by tissue from the uterus (probably from the endometrium. By analogy to more highly differentiated cell types, prostaglandin synthetase, adenyl cyclase, and other plasma membranal enzymes may be anticipated to play important roles in regulation of embryonic differentiation.

PROPOSED COURSE: Completion of the following test will permit identification and recovery of rats which are and are not susceptible to the teratogenic stress associated with maternal consumption of a pantothenate-deficient diet during pregnancy. A minimum of three successive matings are performed using each pairing of animals. Offspring of the first and third matings are subjected to anatomical (teratology) examination, while those from the second, fourth, etc. matings will be retained for further studies. A suitable inbred mouse strain will also be sought.

Feeding studies will be performed to determine whether those animals (and their offspring) which exhibit susceptibility to the teratogenic stress of pantothenate deficiency are also preferentially susceptible to the teratogenic stress(es) imposed by drugs (such as aspirin), by classical teratogens (e.g., trypan blue), and by dietary deficiences involving other essential nutrients (e.g., folic acid or zinc).

If it is possible to obtain an inbred strain of mouse in which teratogenic responsiveness to pantothenate can be staged, it will be possible to perform a genetic linkage study to determine the gene loci involved.

Biochemical correlates to be investigated because of their physiological significance in growth and/or differentiation include the following: (1) Metabolite profiles during pregnancy should reveal differential rates and/or extents of excretion or accumulation (in liver, urine, embryonic tissue, etc.) of pantothenate, pantothenate-dependent compounds, as well as products of regulatory enzymes (e.g., urinary cyclic nucleotides). (2) Regulatory enzymes

associated with the plasma membrane [prostaglandin synthetase, $(Na^{+}+K^{+})$ATPase and/or adenyl cyclase] will be examined in order to obtain more information about behavior of such systems and to determine whether such enzymes might be susceptible to perturbation by teratogenic stress(es) such as pantothenate deficiency, various specific inhibitors, and various known teratogens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 70300-06 ETB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Marsupial Neonate as a Model for the Identification and Evaluation of Environmental Toxicants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Jurgelsky, Jr.	Medical Officer	ETB NIEHS
OTHER:	J. Rice	Head, Perinatal Carcinogenesis Section	NCI NIH
	A. Moore	Chief	MAPB NIH
	P. Mortillaro	Cinematographer	MAPB NIH
	J. Romine	Cinematographer	MAPB NIH
	A. Godwin	Photographer	MAPB NIH
	G. Alderson	Oral Pathologist	AFIP
	C. J. Davis	Renal Pathologist	AFIP
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	L. Johnson	Bone Pathologist	AFIP
	J. M. Henry	Neuropathologist	AFIP
	U. J. Lewis	Chemist	Scripps Institute

COOPERATING UNITS (if any)

Experimental Pathology Branch, Scripps Institute, La Jolla, CA
Med. Arts & Photo. Branch, NIH
Oral, Ophthalmologic, Orthopedic, Hepatic, Renal, Neuropathology, & Pulmonary Branches, AFIP

LAB/BRANCH

Environmental Toxicology Branch

SECTION

Marsupial Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to develop the opossum Didelphis virginiana as a biomedical model by (1) characterizing the normal neonatal and developing opossum anatomically and physiologically and (2) determining the pathophysiologic response of these animals to selected environmental toxins and carcinogens.

PROJECT DESCRIPTION

OBJECTIVES: (1) To develop techniques for efficient breeding of the opossum under laboratory conditions; (2) to characterize the neonatal and developing opossum anatomically and physiologically; and (3) to determine their pathophysiologic responses to selected environmental toxins and carcinogens.

METHODS EMPLOYED: Special pharmacological histologic techniques, light and electron microscopy, and standard biochemical methods of ultracentrifugation, thin-layer chromatography, and gas chromatography are utilized.

MAJOR FINDINGS:

(1) Breeding and Laboratory Management of the Opossum. Paper No. 4 in the series on the opossum titled: "Some Aspects of the Reproductive Behavior of the Opossum (Didelphis virginiana) in Captivity" has been submitted for publication.

(2) Opossum Film. Completion of the opossum films (entitled: "The American Opossum as a Biomedical Model." Part I: "The Neonatal and Developing Opossum" and Part II: "The Adult Opossum") continues to be delayed due to unavailability of resources. NIEHS management has indicated that funds will not be released for the purpose of completing this work until the carcinogenesis work is complete. On this basis, the projected completion date for this project is Fall of 1977 or later.

(3) Biologic Characterization of the Opossum. (a) Studies to isolate and characterize growth hormone and prolactin from the opossum pituitary in collaboration with Dr. U. J. Lewis, Scripps Institute, LaJolla, California, are completed. These hormones were identified and characterized electrophoretically. The data are being prepared for publication. (b) All other studies have been aborted due to withdrawal of NIEHS support which sought to characterize the opossum for the opossum program.

(4) Carcinogenesis Study. (a) Work continues on the characterization of the lesions induced in developing opossums with ethylnitrosourea (100 mg/kg). An additional embryonal tumor, a rhabdomyosarcoma, has been identified.

As with the induced embryonal tumors of the eye (teratoid medulloepitheloma), brain (ganglioglioma), and jaw (myxoma), this neoplasm has not previously been induced by systemic exposure of the traditional laboratory animals to carcinogens.

A tumor of the neuron (ganglioglioma) has been found in eight additional animals. These neurogenic lesions fit a continuum from nearly pure glial tumors to nearly pure neuronal neoplasms.

Data on the incidence, age distribution, and litter distribution of non-embryonal tumors in comparison to embryonal tumors are being summarized for presentation at the Third International Conference on Detection and Prevention of Cancer in April 1976.

Neither the autopsy phase of the pilot study to compare the effects of intraperitoneal and intraocular administration of ENU with the effects of oral administration nor the autopsies in the murine RNA virus study have as yet been completed.

(b) Work to explore possible correlations in the opossum between susceptibility to embryonal tumor induction by ethylnitrosourea and differentiation of the target tissue has been initiated. This work is handicapped by the limited material available (further use of the opossum has been banned within the Environmental Toxicology Branch by Memo of February 13, 1976) and the necessity of working at the light microscopic level. A correlation was apparent to a varying degree between susceptibility to tumor induction and the state of morphologic maturation of the presumed target tissue at the light microscopic level for embryonal tumors of the eye, kidney, and brain. The susceptibility of the opossum eye to an ENU induced teratoid medulloepithelioma extended over the period from 1 to 3-4 weeks of age and was correlated with the differentiation of the apparent target cell, the nonpigmented cilliary epithelium of the pars cilliaris retinae. Induction of nephroblastomas of the kidney was correlated with the presence of stem cells (metanephric blastema) through the period birth to 6-8 weeks of age. Susceptibility of the opossum brain to ENU induction of gangliogliomas was correlated with the state of differentiation of the germinal matrix. No definite correlation between vulnerability to tumor induction and tissue maturation was evident for a tumor of the jaw with presumed origin from embryonic remnants. Nephroblastomas were found in direct association with malformations of the kidney in four animals. Susceptibility to these combined lesions was correlated with the beginnings of functional maturation in the metanephric kidney.

Further work utilizing daily exposure of the opossum to ENU in the late prenatal period and throughout the postnatal period is required to more precisely determine the size of the "window" of vulnerability to each of the four ENU induced embryonal tumors and to the malformation-tumor combinations. Efforts to identify and characterize the mechanisms involved in opening and closing these "windows" will initially require more definitive studies of the correlation between susceptibility to tumor induction and differentiation of the target tissue at the cellular, biochemical, and molecular level.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Our results to date imply that the opossum, early in postnatal life, is a useful model system for the study of certain of the major dysontogenetic neoplasms which have been difficult or impossible to reproduce in the standard laboratory species. The ability to induce embryonal tumors in the opossum may be explained in part by the unique opportunity in this species for the direct exposure of slowly maturing embryonic and fetal tissue to carcinogens in the

absence of interference attributable in the eutherian placental mammal to the mother, the placenta and the fetaplacental unit. A more important factor facilitating the induction of such tumors in this species may be a slow sexual maturation and a relatively late postnatal initiation of endocrine and lymphoid function, possibly providing a longer period than in the eutherian laboratory mammal for the initiation and development of the oncogenic process in the absence of the inhibition often associated with full endocrine and immunologic function.

Our findings dispute the widespread belief that mammals which are lower on the evolutionary scale than primates and rodents make less accurate models for human cancer.

There appears to be a number of advantages of the marsupial model for embryonal carcinogenesis. The opossum model provides an opportunity to directly evaluate the relationship between susceptibility to embryonal carcinogenesis and differentiation of the target tissue. The model may also be of value in exploring the apparent interrelationship among oncogenesis, teratogenesis, and mutagenesis in the absence of the major handicap intrinsic to the eutherian animals, namely, the impossibility of distinguishing direct carcinogen induced teratologic or mutagenic changes from identical lesions which are the indirect result of adverse physical and physiologic effects of the carcinogen on the maternal or fetoplacental unit. In the semiembryonic, semifetal opossum orally or parenterally exposed to a carcinogen, mutations and malformations can only be either carcinogen induced or spontaneous.

PROPOSED COURSE:

(1) Carcinogenesis Study. Histologic preparation of tissues and histopathologic interpretation and classification of lesions will continue as resources allow. The current level of technical support and resources will greatly delay completion of this work for publication.

(2) Opossum Films. Completion of the films will depend on the availability of pregnant opossums and technical and fiscal support.

(3) The Environmental Toxicology Branch has banned further work on the opossum with NIEHS resources. Therefore, no further work on this model will be possible unless the current prohibition is successfully challenged.

PUBLICATIONS

Jurgelski, W., Hudson, P. M., Falk, H. L., and Kotin, P.: Embryonal neoplasms in the opossum: A new model for solid tumors of infancy and childhood. Science (in press).

PHARMACOLOGY BRANCH

PHARMACOLOGY BRANCH
Summary Statement

The Pharmacology Branch initiates and carries out research programs that are primarily concerned with (1) mechanisms by which environmental pollutants and effectors cause toxicity, (2) the development of better test systems for detecting and evaluating early toxic effects of environment chemicals, and (3) extrapolating from animal test systems across species and to man. The Branch is organized into three formal sections and one work group. The sections are Toxication-Detoxication, Marine Pharmacology and Biomedicine, and Pharmacokinetics; the work group is Molecular Pharmacology and Biomedicine.

Program

Toxication-Detoxication Section: This program area is concerned with the role of chemical metabolism in the production of tissue damage and toxicity. Metabolism leading to increased toxicity may be balanced by reactions with detoxifying systems. This balance between toxication and detoxication pathways may be altered by pollutants, other chemicals, pathologic states, physiological changes (age or pregnancy), species and strain differences, hormonal changes, or it may be different in one tissue from another. Chemical-metabolizing systems have been or are being characterized in tissues most likely to contact the environment, i.e., skin, intestine, lung, and liver. Characterization includes substrate and inhibitor specificity, ability to be stimulated or induced, and measurements of components of multicomponent systems (e.g., P-450-dependent mixed-function oxidase (MFO) systems).

The MFO system from rabbit liver and lung has been solubilized and separated into its component parts (cytochrome P-450, NADPH-cytochrome c reductase, and lipid). Assessed are factors responsible for tissue or species differences in substrate specificity for MFO systems. This is being done by comparing enzymatic activities for various substrates after recombination of these components from the different sources.

The balance between toxication and detoxication pathways in lung is being studied and compared with liver using N-demethylation, N-hydroxylation, and N-oxidation as model systems. N-demethylation usually is a detoxifying reaction, while N-hydroxylation and N-oxidation result in more toxic metabolites. A P-450 independent system for N-oxidation has been solubilized and partially purified from liver and lung. Kinetic studies have suggested that liver and lung N-oxidases may be different enzymes. Further, levels of N-demethylation and N-oxidation activities in lung and the effects of steroids on these activities have been followed at different stages of fetal life as well as in pregnant vs. nonpregnant animals. Studies of changes caused by pregnancy or steroids in toxication vs. detoxication pathways may aid our identifying and understanding stages in life when the body is more susceptible to toxic agents.

In skin, the MFO system seems especially unlike liver in its response to polycyclic hydrocarbon inducers as well as in its concurrent content of non-P-450 hemoproteins. These various studies may help explain the sensitivity of skin and lung to certain types of chemicals whose toxic effects depend on metabolic activation.

The xenobiotic-metabolizing enzyme systems in intestine were characterized. In studies on various animal species, rabbit was shown to be the best animal model for study of xenobiotic metabolism(s) in small intestine as enzyme activity for all pathways studied was present in easily measurable quantities. Studies of the postnatal development of xenobiotic-metabolizing enzymes and cytochrome P-450 in the small intestines and livers of rabbits showed that, in general, the patterns of development of these enzymes in small intestine were similar to those seen in the liver. However, unlike liver enzymes, the intestinal enzymes failed to respond to pretreatment by inducers such as DDT, TCDD, 3-MC, and phenobarbital.

Metabolic activation of chemicals as a key process in toxicity was the subject of a three-day conference cosponsored with the Environmental Mutagenesis Branch, the Drug Metabolism Division of the American Society for Pharmacology and Experimental Therapeutics, and the American Environmental Mutagen Society. The conference brought together leading researchers in both chemical metabolism and mutagenesis and attempted to acquaint workers in both fields with the state of the art and needed studies on the role of chemical metabolism in producing genetic damage.

Marine Pharmacology and Biomedicine: This program area conducts studies on uptake, distribution, metabolism, and excretion of pollutants by various marine species, and the role of metabolism of these pollutants in their storage and storage forms in these species. The major emphasis is on how, why, and in what tissues and organs marine species accumulate pollutants which may be dangerous to man and whether mixtures of pollutants may lead to accumulation of more toxic levels or forms of pollutants than single chemical exposure. Year round operation of a new laboratory (Whitney Marine Laboratory, St. Augustine, Florida) allows studies which expand and complement those which have been carried on in our summer-only laboratory (Mount Desert Island Biological Laboratory, Maine). For example, comparisons are being made of the effect of water temperature on metabolic and storage processes for pollutants.

The pharmacokinetics of pollutant distribution in selected marine species is being determined and compared with mammals (in collaboration with the Pharmacokinetics Section). So far we have shown that a polychlorinated biphenyl (PCB) isomer is stored in different tissues in different marine species and that the biological half-life of PCB correlates with the ability of the species to metabolize it. Compared with mammalian systems, marine species have a relative deficit in the capability to carry out oxidative chemical metabolisms, while possessing adequate levels of enzymes for conjugation reactions. Thus, we have shown that water soluble pollutants, such as 2,4-D and 2,4,5-T, are rapidly conjugated with amino acids and excreted

in urine and bile while liposoluble pollutants such as DDT or PCB are often accumulated. Currently, the dispositions of aliphatic and aromatic constituents of crude oil are being investigated.

Investigations are being made of the biotransformation of various organic pollutants in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Particular emphasis is being placed on studying formation and further metabolism of chemically reactive metabolites (e.g., from hydrocarbons which are constituents of crude oil). Both cytochrome P-450-dependent microsomal mixed-function oxidases and epoxide or arene oxide metabolizing enzymes (epoxide hydrase and glutathione S-transferases) are being characterized in control marine species and in species pre-exposed to environmental contaminants such as polycyclic aromatic hydrocarbons or dioxins. Where biologically significant induction was observed, the induced system is being characterized in considerable detail, e.g., in little skates.

In a separate, but complementary, program of the Section, we are studying the formation and metabolism of reactive metabolites of various hydrocarbons, especially epoxides and arene oxides, in hepatic and extrahepatic tissues of several mammalian species. This effort involves considerable collaboration with the Toxication-Detoxication Section as well as with researchers outside NIEHS (Laboratory of Chemistry and Laboratory of Biochemistry and Metabolism, NIAMDD). Several epoxides and arene oxides are known to react covalently with macromolecules, including nucleic acids and proteins, suggesting their role as ultimate carcinogens, mutagens, and cytotoxins. We are studying the further metabolism of epoxide and arene oxides by soluble fraction glutathione S-transferases and microsomal epoxide hydrase in liver, lung, kidney, testes, and intestinal mucosa of adult and immature rats, rabbits, and guinea pigs. The relative importance of the two pathways for selected epoxides and arene oxides is being assessed after pretreatment with other chemicals and with use of the isolated perfused liver and lung preparations. Substrate specificities for reactive metabolites are being measured in different tissues and at different times of life since differences in substrate specificity may explain why certain tissues are more sensitive than others to the toxic effects of these metabolites.

The Section is also involved in developing a national program in marine pharmacology as directly applicable to human health problems (models for human disease, sources of toxic materials for man). The main direct concern of the NIEHS will be to provide a focus for DHEW activities in marine biomedicine.

Pharmacokinetics Section: This section is conducting research in at least two major categories: (1) whole body distribution of pollutants and formulation of pharmacokinetic models for extrapolation, and (2) uptake, storage, metabolism, and release of pollutants and model compounds by isolated perfused organs, with present emphasis on lung and liver. The kinetics of chemical disposition in intact animals and isolated organs are being determined in order to construct mathematical models of these processes which can be used:

(a) to better define and understand rate-limiting steps in the process, (b) to extrapolate distribution profiles of chemicals from one tissue to another, one species to another, and to man, and (c) to predict tissue storage of pollutants from knowledge of routes, dose, and numbers of exposures. Comparisons of the kinetics of pollutant distribution in mammalian vs. marine species are being carried out in collaboration with Marine Pharmacology and Biomedicine, as previously mentioned.

Through extensive collaboration with the Environmental Biometry Branch and the Biomedical Engineering and Instrumentation Branch, DRS, NIH (Drs. R. L. Dedrick and R. Lutz), pharmacokinetic models have been developed for four PCB isomers in the rat. These models can predict disposition of the PCBs for up to 42 days after a single i.v. dose. The biochemical and physiological parameters used in this model were determined from studies in bile cannulated animals, isolated perfused liver studies, and whole animal disposition studies. Currently, these parameters are being studied in the mouse and extended to two additional isomers in both rats and mice. Major findings on the sites and patterns of disposition of PCBs have ben reported. Recent studies support the hypothesis that two adjacent unsubstituted carbon atoms on one of the biphenyl rings are necessary for an appreciable rate of metabolism and subsequent excretion of the PCBs. The major metabolites of the PCBs were shown to be mono- and dihydroxylated derivatives which are usually excreted as glucuronide conjugates. Collaborative studies are just underway through the contract mechanism which will provide information on the disposition of lipophilic environmental chemicals in man. These studies concern the pharmacokinetics and toxicity of the pesticide, Kepone, in humans and similar studies with the polybrominated biphenyls.

Another continuing major effort of the Section is in examination of pharmacokinetic functions of the lung with regard to both exogenous and endogenous chemicals. These studies involve the use of the isolated perfused rat and rabbit lung preparations as research tools. With regard to exogenous chemicals, detailed models were developed for the dynamic interaction of the perfused lung with circulating basic amines. The persistence in lung tissue of certain basic amines was studied both in vitro and in vivo. This non-effluxable pool of amines was thought to be associated with phospholipid complexes in concentric lamellar organelles, and, thus, these amines may be involved in the production of pulmonary phospholipidosis. Examination of the non-effluxable pool may permit development of methods for predicting or modifying chemical-induced lipidoses in lung tissue. Other studies dealt with the uptake and persistence of the herbicides, paraquat and diquat, in the perfused rabbit lung. Differences in the amount and persistence of paraquat and diquat in lung may correlate with the difference in the pulmonary toxicity of these two herbicides. With regard to endogenous chemicals, a pharmacokinetic model was developed for the serotonin (5-HT) detoxification system. From this model, it was evident that flow rates are important in examining transport systems in isolated organs. In general, elucidation of the rates and mechanisms of lung uptake, storage, and metabolism of

chemicals may allow prediction of possible toxic interactions between environmental pollutants and other chemicals, including drugs and endogenous chemicals such as 5-HT.

Another group of endogenous compounds regulated by the lung are the hormones known as the prostaglandins. These lipids are synthesized, stored, and metabolized in lung, and the lung plays a very important role in regulating circulating levels of these very active materials. Effects of pollutants (oxidant gases) on prostaglandin (PB) disposition by the lung are being investigated. Effects seen are usually on the metabolism of PGs rather than on their biosynthesis. The relationship between depression of PG metabolism and decrease of pulmonary Type I cells resulting from oxidant gas exposure may be useful in development of an early warning system for pulmonary damage. Certain PGs are accumulated in lung by a carrier-mediated transport process, while other PGs and metabolites are not. We are currently characterizing the structural requirements for a PG to be taken up by the transport system. Collaboration with chemical researchers in the Department of Medicine, Duke University, is enabling us to compare our findings on PG systems in animals to studies in humans with pulmonary diseases.

Molecular Pharmacology and Biochemistry Work Group: This group is involved in studies which can be categorized under either: (1) pharmacological and biochemical research on synthesis, metabolism, release, isolation, and identification of endogenous chemicals (lipids, proteins, enzymes) involved in lung function, both in normal and in disease states. Information is being used to develop markers for early detection of lung disease, and correlation is being made with human disease states of possible environmental origin, or (2) studies on chemical-macromolecule interactions, particularly those that are associated with cell membranes.

In the program investigating pulmonary alveolar secretions attention has been focused on the isolation and characterization of the concentric laminar organelle (CLO) of the alveolar Type II cell. The CLO is a precursor for the acellular surfactant material lining the alveoli. Other studies have focused on enzymes in the lining layer which are specific to lung as opposed to other tissues. Soluble alkaline phosphatases have been characterized and appear to exhibit such specificity. In conjunction with researchers at Duke University, we are attempting to relate these studies to human pulmonary secretory diseases, with emphasis on two diseases of unknown etiology which may have an environmental component; namely, alveolar proteinosis and bronchial asthma. We have shown the presence of an abnormal, high molecular weight alkaline phosphatase complex in lavage effluents of patients with alveolar proteinosis. The presence of this complex in these samples may aid in diagnosis of this disease. In another study, the presence and nature of pulmonary specific components in human amniotic fluid are being evaluated as potential markers for fetal lung maturity.

Studies are being made of membrane peptides and glycopeptides in normal liver and in transplantable hepatomas derived from animals treated with chemical carcinogens. We have investigated the loss of hormonal (glucagon)

stimulation of adenylate cyclase in plasma membranes in certain hepatomas. In another study, we have investigated a specific glycoprotein, NAD glycohydrolase, isolated from endoplasmic reticulum and plasma membranes of liver. We are attempting to establish specific compositional and structural changes occurring in membranes of carcinogen-induced tumors. Some membrane glycoproteins are thought to play a key role in the growth regulation of cells. In a recently initiated study, polypeptides secreted by lung tumors are being studied as possible early markers for the development of such tumors, and in vitro studies with chemical carcinogens will attempt to correlate development of neoplasia with production of specific polypeptides (such as ACTH) as markers for neoplasia.

Personnel

Two major personnel shifts took place during the year. Dr. J. R. Fouts (Branch Chief) moved up to the post of Acting Scientific Director of the Institute at about mid year and Dr. L. G. Hart (Assistant Branch Chief) was appointed Acting Chief of the Branch. As part of an Institute reorganization, the Aerotoxicology Section moved into the Environmental Biology and Chemistry Branch. Personnel moving with the Section included Dr. R. Drew, Section Head, Dr. J. O'Neil, Dr. E. W. Van Stee, Dr. J. M. Patel (Visiting Fellow), Mr. W. Gibson, Mr. F. Harrington, Mr. M. Riley, Ms. J. Schroeder, and Ms. K. Wade.

Additions to the Branch were--Visiting Scientists--Dr. A. Ryan (with Dr. Bend); Visiting Associate--Dr. A. Aitio (with Dr. Bend); Visiting Fellows--Dr. A. Chaudhari (with Dr. Eling), Dr. M. Kahn (with Dr. Bend), Dr. I. Linnoila (with Dr. DiAugustine), Dr. C. Miranda (with Dr. Chhabra), Dr. N. Morales (with Dr. Matthews), and Dr. D. Nadeau (with Dr. Hook); NIH Postdoctoral Fellows--Dr. M. Reasor (with Dr. Hook), Dr. B. Smith (with Dr. Philpot). New technicians were Mr. J. Brittian (with Dr. Eling, replaced Ms. Guthrie), Mr. R. Nardone (with Dr. Chhabra, replaced Ms. E. Smart), and temporary technician Mr. G. Harrison (with Dr. James).

Other losses from the Branch included Dr. C. Harper (Senior Staff Fellow), Dr. H. Mehendale (Staff Fellow), Dr. A. Ryan (Visiting Scientist), Dr. A. Aitio (Visiting Associate), Dr. R. Mirel (Staff Associate), Visiting Fellows--Dr. T. Abe, Dr. E. Arinc, Dr. M. Kahn, and Dr. L. Jao; technicians--Ms. D. Eckberg (technician with Dr. DiAugustine), Ms. J. Guthrie (technician with Dr. Eling), Ms. E. Smart (technician with Dr. Chhabra), and Mr. G. Harrison (temporary technician with Dr. James).

Other Activities

Several of our professional staff serve on committees and give lectures or seminars at local universities or help with committees in other Federal agencies. The list below is not inclusive:

Dr. J. R. Bend: An organizer of the NIEHS cosponsored Conference on 'In Vitro Metabolic Activation,' Burroughs Wellcome Auditorium, Research Triangle Park (February 1976); invited speaker at the conference; invited speaker at the

WHO-NIEHS sponsored Symposium on Potential Health Hazards from Technological Developments in the Rubber and Plastic Industries, NIEHS (March, 1976); presented seminars at Texas College of Osteopathic Medicine, North Texas State University, Denton, Texas (February, 1976); and at Department of Entomology, North Carolina State University, Raleigh, N.C. (March, 1976).

Dr. R. S. Chhabra: Member of the Library Committee, NIEHS.

Dr. R. P. DiAugustine: Adjunct assistant professor, Department of Medicine, Duke University; invited lecturer at Department of Pathology, Duke University; member of NIEHS Medical Sciences Lectures Committee.

Dr. T. E. Eling: Invited participant in 'Symposium on the Role of Prostaglandins in Lung Diseases' sponsored by NHLI at Warrenton, Virginia (January, 1976); presented seminar (with Dr. Anderson, EBB) at Departments of Medicine and Biochemistry, Duke University (October, 1975); invited speaker at 'Symposium on Pharmacokinetic Functions of Lung,' Turku, Finland (july, 1975).

Dr. L. G. Hart: Adjunct associate professor, Department of Pharmacology, University of North Carolina, lectures presented to medical and graduate students of University of North Carolina; member of NIEHS Safety Committee; NIEHS project director for coordinating efforts in three task areas under the "health effects of the non-nuclear energy technologies" program; associate managing editor (U.S.) for Chemico-Biological Interactions; editorial board member of Environmental Health Perspectives.

Dr. G. E. R. Hook: Adjunct assistant professor, Department of Medicine, Duke University; co-managing editor and editorial board member of Environmental Health Perspectives.

Dr. H. B. Matthews: Chairperson of group reporting on the metabolism and biochemical toxicology of PCBs to the FDA Subcommittee on the Health Effects of PCBs.

Dr. R. M. Philpot: Adjunct assistant professor, Department of Entomology, North Carolina State University; member of NIEHS editorial board; an organizer of the NIEHS co-sponsored conference on "In Vitro Metabolic Activation,' Burroughs Wellcome Auditorium, Research Triangle Park (February, 1976); presented seminar at Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee (June, 1975).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80001-05 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Microsomal Mixed-Function Oxidase Systems: Specificity and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. M. Philpot	Research Chemist	PB	NIEHS
OTHER:	Emel Arinc	Visiting Fellow	PB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pharmacology Branch

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this research project is to assess the factors responsible for differences in the substrate specificities among cytochrome P-450-dependent microsomal mixed-function oxidase systems (MFO) from various sources. Present work involves the purification of cytochrome P-450, NADPH-cytochrome c reductase, and lipid fractions from rabbit pulmonary and hepatic microsomes and from little skate (Raja erinacea) hepatic microsomes. The cytochrome P-450s are being examined by UV-visible spectroscopy, electron paramagnetic resonance spectroscopy, SDS-gel electrophoresis, and by activities in reconstituted systems. The long range objective of this work is to determine the influence of: 1) multiple forms of the enzymic components of the MFO system; 2) endogenous compounds; and 3) exogenous compounds (inhibitors and inducers) on the substrate specificities of MFO systems from different tissues and species.

PROJECT DESCRIPTION

OBJECTIVES: The microsomal mixed-function oxidase (MFO) system of mammals exhibits species, sex, and tissue variations. Treatment of animals with various pharmaceutical agents, environmental pollutants, and other exogenous compounds can bring about changes in the MFO system(s). Both stimulation and inhibition of these systems can be elicited, and in some cases this can be accomplished with the same compound. It is not understood if these changes are due to direct effects on the major components of the MFO, to alterations in the membrane environment, or to changes in the concentrations or kinds of endogenous compounds that interact with the MFO.

The objective of this project is to increase our understanding of the factors responsible for the variances in substrate specificity and activity noted among mixed-function oxidase systems from different sources. This objective is being pursued via several approaches:

1. Tissue Differences. The MFO systems from rabbit liver and lung are being solubilized and separated into their component parts. These components (cytochrome P-450, TPNH cytochrome c reductase and lipid) and the enzymatic activities obtained by component recombination are being compared.

2. Cytochrome P-450 Differences. Rabbit pulmonary and hepatic cytochrome P-450's are being purified and compared.

3. Stain Differences. The hepatic MFO systems of various strains of rabbits respond differently to in vivo treatment with polycyclic hydrocarbons. The components of the pulmonary and hepatic MFO systems from two strains of rabbits are being examined before and after such treatments.

4. Species Differences. The hepatic MFO system from the little skate, Raja erinacea, is being compared to the mammalian system. The response of this marine animal to an inducer appears to be markedly different from that observed in mammals. The reasons for this difference are being determined.

METHODS EMPLOYED: The components of the liver MFO systems are solubilized and separated by the methods developed by Lu and co-workers. These methods have been modified for application to the rabbit pulmonary MFO system. Pulmonary cytochrome P-450 is being purified by the combination of existing techniques for liver cytochrome purification and the development of new techniques. Spectral analysis of cytochrome fractions is carried out according to methods developed in this laboratory.

MAJOR FINDINGS: 1. The Rabbit Pulmonary MFO System. Cytochrome P-450 has been purified 32-fold from rabbit pulmonary microsomes. The cytochrome is spectrally similar to hepatic cytochrome P-450. Preparations containing 7-8 nmoles cytochrome P-450 per mg protein do not contain any cytochrome b_5 or NADPH-cytochrome c reductase. However, epoxide hydrase has been found

to co-purify with cytochrome P-450. NADPH-cytochrome c reductase prepared from lung by the methods used with liver does not support hydroxylase activity when combined with pulmonary cytochrome P-450 but is active in combination with hepatic cytochrome P-450.

2. The Rabbit Hepatic MFO System. Two forms of cytochrome P-450 have been purified from hepatic microsomes prepared from untreated rabbits. One form (P-448) resembles the cytochrome induced by polycyclic hydrocarbons. The relationship between cytochrome P-448 from treated and untreated rabbits is being investigated.

3. The Little Skate, Raja erinacea, Hepatic MFO System. The hepatic MFO system from the little skate has been solubilized and separated with its component parts: cytochrome P-450, NADPH-cytochrome c reductase and lipid. The cytochrome has been purified to 3.0 nmoles per mg protein and has been combined with rabbit NADPH-cytochrome c reductase to reconstitute hydroxylase activity.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A full understanding of mixed-function oxidase systems is required if drug interaction, drug metabolism, disposition of ingested or inhaled environment pollutants, and pesticide detoxification are to be fully comprehended.

This problem is complicated by the multitude of functional differences and responses exhibited by MFO systems from various sources. Identification of the factors which control the activities of MFO systems is essential to our understanding and manipulation of this complex biological process.

The methods by which the lung deals with inhaled substances are of particular importance. The lung is a target organ for the action of polycyclic hydrocarbons which are an important class of environmental pollutants. It has been hypothesized that these compounds must be metabolized by the MFO system in order to become effective as carcinogens and/or mutagens. The differences between the lung and liver systems in dealing with these compounds may provide part of the explanation for target organ specificity.

PROPOSED COURSE: The present work involving the solubilization, reconstitution, and determination of substrate specificities of the MFO systems from rabbit liver and lung will be continued. The effect of 3-methylcholanthrene in rat and rabbit will be further examined in an effort to identify the factors responsible for the differences noted in responses. Efforts to purify cytochromes from the two tissues will continue. Immunological studies are being initiated with Dr. Faith (EBCB) and EPR studies with Dr. Rajagopalan (Duke University). Subunit molecular weight determinations are being done jointly with Ms. D. Bell (NCSU).

PUBLICATIONS

Philpot, R. M., Arinc, E., and Fouts, J. R.: Reconstitution of the rabbit pulmonary microsomal mixed-function oxidase system from solubilized components. Drug Metab. Disp. 3: 118, 1975.

Philpot, R. M. and Arinc, E.: Separation and purification of two forms of hepatic cytochrome P-450 from untreated rabbits. Molec. Pharmacol. In press, 1976.

Arinc, E. and Philpot, R. M.: Preparation and properties of partially purified pulmonary cytochrome P-450 from rabbits. J. Biol. Chem. In press, 1976.

Philpot, R. M. and Arinc, E.: Solubilization, separation, and partial purification of cytochrome P-450 and cytochrome b_5 from hepatic microsomes of the little skate, Raja erinacea. Bull. Mt. Desert Island Biol. Lab. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80002-06 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Enzymes Metabolizing Chemicals: Chemical and Physiological Effectors of These Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. R. Devereux	Research Biologist	PB	NIEHS
	J. R. Fouts	Acting Scientific Director		NIEHS
OTHER:	R. M. Philpot	Research Chemist	PB	NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch, Environmental Biology and Chemistry Branch

LAB/BRANCH
Pharmacology Branch

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range purpose of this project to study how various chemicals (pollutants, steroids, etc.) and physiological changes (age, pregnancy, etc.) affect xenobiotic metabolism by the body. This laboratory has concentrated its effort on the lung as a target organ for exposure to environmental stresses. The balance between detoxication and possible toxication pathways in lung is being studied using N-demethylation, N-hydroxylation, and N-oxidation as model systems. The topics of present interest are the following: 1) hormonal and steroid effects on fetal and neonatal microsomal N-oxidase and N-demethylase activities in lung; 2) N-hydroxylation of a primary amine versus O-deethylation of a non-N-containing compound by a purified and reconstituted lung mixed-function oxidase (MFO) P-450 system to see if differences exist which might be linked to the toxicity of the N-hydroxylation reaction; and 3) N-oxidation of secondary and tertiary amines in rabbit liver microsomes and purified enzyme systems to see if two enzymes are involved in these two reactions. Studies in the near future will include the isolation of lung cell types to study localization of xenobiotic metabolism within the lung and to study toxication-detoxication mechanisms of individual cell populations.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: This effort is directed towards understanding how various chemicals (pollutants, steroids, etc.) and physiological changes (age, pregnancy, disease) affect the way xenobiotics are metabolized by the body. Using N-demethylation, N-hydroxylation, and N-oxidation as model systems, detoxication and possible toxication-metabolic pathways of the same substance are compared. Present efforts are devoted to studies of hormonal and steroid effects on fetal and neonatal lung microsomal N-oxidase and demethylase activities. N-hydroxylation of a primary amine (p-chloroaniline) versus O-deethylation of a non-N-containing compound (7-ethoxycoumarin) by a purified and reconstituted rabbit lung mixed-function oxidase (MFO) system is being studied to see if differences exist which might be linked to the toxicity of the N-hydroxylation reaction. N-oxidation of secondary (p-chloromethylaniline) and tertiary amines (dimethylaniline) in rabbit liver microsomes and purified systems is being studied to see if more than one enzyme might be involved in these two reactions.

METHODS EMPLOYED: Most work has been done on microsomal preparations from liver and lung of rabbits. These microsomes have been used to study the metabolism of different xenobiotics or the amount or activity of key steps in the MFO-P-450-dependent system as well as the P-450 independent N-oxidation system. The N-oxidase enzyme has been partially purified from liver and lung by classical techniques of enzyme purification--solubilization with detergents, concentration by selective precipitation with $(NH_4)_2SO_4$, separation on DEAE-cellulose columns, etc. Spectrophotometric and fluorometric methods are used to study cytochrome P-450 and to quantify metabolites of substrates added to microsomes or purified enzyme systems.

MAJOR FINDINGS: Increased levels of N-demethylase and N-oxidase activities were seen in 27-day fetal lung microsomes of rabbits after the mothers were treated with the corticosteroid dexamethasone on days 24, 25, and 26 of pregnancy. However, no change in drug metabolizing activities were seen in 24-day fetal lung microsomes following treatment of the mother with the corticosteroid on days 21, 22, and 23 of pregnancy. The solubilized and partially purified rabbit liver and lung N-oxidases were studied to learn why Hg^{+2} stimulated the microsomal lung N-oxidase but inhibited the N-oxidase in liver microsomes. An "activation factor" isolated from liver and lung was found to effect Hg^{+2} stimulation of the N-oxidase in lung by lowering the effective Hg^{+2} concentration. Kinetic studies showed that the liver and lung N-oxidases differed with respect to their response in vitro to Hg^{+2}. This suggests that the liver and lung N-oxidases may be different enzymes.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These research projects seek to understand some of the factors that affect xenobiotic metabolism in tissues in contact with the environment. Comparisons of lung and liver systems have been made to see where differences exist and what these might mean in toxication-detoxication mechanisms of the body. Studies of changes caused by pregnancy or steroids in toxication versus detoxication pathways may aid our identifying and understanding stages in life

when the body is more susceptible to toxic agents. These studies, as well as those into the mechanisms of action of metal ions on N-oxidation in lung and liver and the comparisons of similar enzyme systems (e.g., N-hydroxylation vs. N-oxidation) in different tissues, may lead us to a better understanding of the balance between toxication and detoxication mechanisms and the varied ways chemicals and physiological changes can alter these systems and this balance.

PROPOSED COURSE: Future work on this project will involve isolation of rabbit lung cells and culture of individual cell lines in order to localize xenobiotic metabolism in the lung to specific cell types. Future studies will be undertaken in an effort to further understand detoxication-toxication mechanisms and the ways chemical and physiological stresses alter these systems within the cell.

PUBLICATIONS

Devereux, T. R. and Fouts, J. R.: Effect of pregnancy or treatment with certain steroids on N,N-dimethylaniline demethylation and N-oxidation by rabbit liver or lung microsomes. Drug Metab. Disp. 3: 254-258, 1975.

Bend, J. R., James, M. O., Devereux, T. R., and Fouts, J. R.: Toxication-detoxication systems in hepatic and extrahepatic tissues in the perinatal period. In Basic and Therapeutic Aspects of Perinatal Pharmacology. Ed. by Morselli, P. L., Garattini, S., and Sereni, F., New York, Raven Press, 1975, pp. 229-243.

Pan, H. P., Fouts, J. R., and Devereux, T. R.: Hepatic microsomal N-oxidation and demethylation of N,N-dimethylaniline in red-winged blackbird compared with rat and other birds. Life Sciences 17: 819-826, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80003-03 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Xenobiotic Metabolizing Enzyme Activity in Skin and Its Response to Environmental Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Pohl	Research Biologist	PB	NIEHS
	J. R. Fouts	Acting Scientific Director		NIEHS
OTHER:	R. M. Philpot	Research Chemist	PB	NIEHS
	J. R. Bend	Visiting Associate	PB	NIEHS

COOPERATING UNITS (if any)
Environmental Biometry Branch

LAB/BRANCH
Pharmacology Branch

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.8	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to elucidate the role of xenobiotic metabolizing enzymes in toxic responses of skin to environmental agents. Enzyme activity and cytochrome P-450 content are assayed in skin microsomes isolated from hairless mice and NZW rabbits after exposure of the animals to polycyclic hydrocarbons, chlorinated hydrocarbons, ultraviolet radiation, etc.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To isolate microsomal fractions from skin, characterize the xenobiotic metabolizing enzymes associated with those fractions, and elucidate the role of these enzymes in toxic responses of skin to environmental agents.

METHODS EMPLOYED: Mouse and rabbit epidermis were disrupted in 0.5 gm aliquots by two 5-second bursts with a Polytron cell disruptor and homogenized in a conical homogenizer. Microsomal fractions were isolated by standard procedures. Cytochrome P-450 content was estimated from reduced CO difference spectra. Contamination by other hemochromes was identified by a variety of spectral difference techniques. 7-Ethoxycoumarin deethylating activity was measured by recording the appearance of the fluorescent metabolite, umbelliferone. Benzpyrene and biphenyl hydroxylase activities were measured by standard techniques. Cytochrome oxidase activity was estimated by measuring oxygen consumption with a Clark electrode. GSH-S-epoxide transferase and epoxide hydrase activities were assayed radiochemically.

MAJOR FINDINGS: Skin microsomal benzpyrene hydroxylase and 7-ethoxycoumarin deethylase activities from hairless mice and Dutch-belted rabbits were increased after topical treatment with TCDD. Both New Zealand white and Dutch-belted rabbits had only trace benzpyrene hydroxylase and 7-ethoxycoumarin deethylase activity in skin microsomes from control animals. Exposure of New Zealand white rabbits to ultraviolet radiation of 254 nm, 300 nm, or 355 nm produced no increase in benzpyrene hydroxylase, 7-ethoxycoumarin deethylase, or glutathione-S-epoxide transferase activity at 30 minutes or 1 to 8 days after exposure.

Hairless mouse skin microsomal biphenyl hydroxylase activity was low, 0.01 to 0.1 nmoles 4-OH- or 2-OH-biphenyl formed/min/mg protein. No increases in skin microsomal benzpyrene hydroxylase activity, 7-ethoxycoumarin deethylase activity, or cytochrome P-450 content occurred 30 min or 1 to 8 days after exposure of hairless mice to 300 nm UV radiation or 4 days after intraperitoneal exposure to Arochlor 1260, 2,4,5,2',4',5'-hexachlorobiphenyl or 3-methylcholanthrene.

Hairless mouse skin and NZW rabbit epidermal glutathione-S-epoxide transferase activity in vitro toward styrene oxide and benzpyrene-4,5-oxide were characterized. No change in activity occurred after exposure to UV radiation or hydrocarbons. Microsomal epoxide hydrase activity in control hairless mouse skin and NZW rabbit epidermis was below measurable limits. Cytochrome oxidase activity per mg protein was the same in liver and skin microsomes from hairless and NZW rabbits.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Skin comes in contact with a large number of foreign chemicals found in cosmetics, cleaning compounds, polluted air and water, synthetic and "easy-care" fabrics, and some occupational environments. Both localized and systemic toxicity of these chemicals may be mediated by the balance of toxicating and detoxicating mechanisms in the skin, including xenobiotic metabolizing enzymes.

The study of these enzymes and their responses to both chemical and physical factors will lead to a better understanding of the total body protective systems toward foreign chemicals.

PROPOSED COURSE: More sensitive techniques to measure mixed-function oxidase (MFO) activity in skin will be sought in order to facilitate measurement of enzyme inhibition. A systematic study will be made of the effects of MFO inhibitors and inducers (many of which are environmental contaminants), age, sex, and physiological stresses on skin enzyme activities and their response to environmental chemicals and radiation of various types.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80004-04 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Study of Factors Affecting Biotransformation of Xenobiotics in Extrahepatic Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. S. Chhabra	Senior Staff Fellow	PB	NIEHS
OTHER:	J. M. Tredger	Visiting Fellow	PB	NIEHS
	J. R. Fouts	Acting Scientific Director		NIEHS

COOPERATING UNITS (if any)
Environmental Biometry Branch, Environmental Biology and Chemistry Branch

LAB/BRANCH
Pharmacology Branch

SECTION
Toxication-Detoxication

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the effects of environmental pollutants such as heavy metals, pesticides, and carcinogenic polycyclic hydrocarbons on the biological functions of the intestine. In past years, the emphasis was on studying the factors influencing the toxication-detoxication of foreign chemicals by intestinal mucosa. Other intestinal functions such as digestion, absorption, and metabolism of nutrients which may be affected adversely by the presence of environmental pollutants in food are under initial stages of investigation.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To study the effects of various physiological (age, sex, nutrition, hormones), genetic (animal species, strain), and environmental (exposure to insecticides, carcinogenic polycyclic hydrocarbons, and heavy metals) factors on the enzymes metabolizing xenobiotics in various tissues.

There are three subprojects currently under study: 1) comprehensive studies of xenobiotic metabolizing enzyme systems in rabbit small intestine and comparison with similar systems in liver and effects of environmental pollutants on these systems; 2) genetic regulation of induction of xenobiotic metabolizing enzymes in various strains of mice; and 3) study of possible circadian variations in the activity of xenobiotic metabolizing enzymes in rabbit liver, small intestine, and lung.

METHODS EMPLOYED: Microsomal fractions were isolated by differential centrifugation. Spectrophotometric and spectrophotofluorometric techniques were used for the assays of xenobiotic metabolizing enzymes.

MAJOR FINDINGS: 1. Various laboratory animal species were used to select a model animal for more detailed study of xenobiotic metabolizing enzymes in small intestine. In intestine from mice, rats, guinea pigs, and hamster, either some of the enzymes were absent or had very low activity which would require very sensitive methods for detection. Rabbit emerged as the best animal model to study xenobiotic metabolism in small intestine because all xenobiotic metabolizing enzymes studied were present in easily measurable quantities. A distribution study of some of the xenobiotic metabolizing enzymes along the entire length of small intestine showed that maximum activity of these enzymes is located in the proximal 24 inches of the small intestine. Rabbit intestinal xenobiotic metabolizing enzymes required reduced nicotinamide adenine dinucleotide phosphate and oxygen for maximum activity. In general the protein vs. activity, time vs. activity, substrate concentration vs. activity, and pH vs. activity curves of aniline hydroxylase, benzpyrene (BP) hydroxylase, and aminopyrine N-demethylase were similar in microsomes from small intestine and liver. Cytochrome c, SKF 525-A, and CO inhibited microsomal mixed-function oxidases in small intestine as well as in liver.

Studies of the postnatal development of xenobiotic metabolizing enzymes and cytochrome P-450 in the small intestines and livers of rabbits showed that, in general, the patterns of development of drug metabolizing enzymes in the small intestine were similar to those seen in the liver. Some of the enzymes studied were detectable during the first week after birth and by the end of the second week all enzymes could be assayed. During the third week after birth, small increases in the activities of the enzymes were observed, but amounts remained less than 50% of those found in the adult. However, 4 weeks after birth, 2- to 5-fold increases in activity occurred and at this time equaled or exceeded those activities in the adult small

intestine. For the next 7 weeks the activities of the intestinal enzymes fluctuated (usually falling below adult values at about 40 to 50 days post-partum), then rising again to adult levels by 7 to 11 weeks of age. The maturation of xenobiotic metabolizing enzyme activity was accompanied by a proportional change in the content of cytochrome P-450 in intestinal microsomes. Hemoglobin from adult or newborn rabbits did not catalyze aniline metabolism. Thus, the relatively high aniline hydroxylase of newborn liver was not due to contamination by hemoglobin.

The rabbits were treated with DDT, tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene (3-MC), and phenobarbital for varying periods of time. These inducing agents failed to alter the xenobiotic metabolizing enzymes in intestine.

2. Various microsomal enzyme inducers such as DDT, benzpyrene, 3-MC, TCDD, or phenobarbital were studied for their effects on hepatic microsomal mixed-function oxidases and cytochrome P-450 content in mice genetically responsive (C57BL/6J) and resistant (DBA/2J) to induction by BP of aryl hydrocarbon hydroxylase (AHH, benzpyrene hydroxylase). Benzpyrene or 3-MC administration to mice stimulated hepatic AHH activity 2- to 3-fold in C57BL/6J but not in DBA/2J mice. TCDD stimulated AHH activity in liver of both mouse strains 6- to 8-fold. This increase was accompanied by a delayed shift in the peak of cytochrome P-450-CO-difference spectrum from 450 to 448 nm. This study suggests that genetic resistance of AHH to stimulation in DBA/2J mice is influenced by the type of inducer used.

3. To facilitate the planned studies of possible (a) circadian rhythms in rabbit mixed-function oxidase activities and (b) to study the toxication-detoxication system in human tissues obtained during surgery, a method for storage of tissue preparations was developed. By freezing and storing, whole tissues or microsomal components isolated from the liver, lung, and small intestine of rabbits could be successfully preserved. The components studied included various xenobiotic metabolizing enzyme activities, NADPH-cytochrome c reductase and UDP-glucuronyltransferase activities, cytochrome P-450 concentrations, and the magnitude of the type I and type II spectral interactions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Species and strain differences in metabolism and response to pollutants by xenobiotic metabolizing systems may explain both species and strain specific toxicities of pollutants and interactions of pollutants with drugs and chemicals. Pollutants in food and water, as well as in air contact intestine, and intestinal enzymes may be a first line of defense of the body against such materials.

PROPOSED COURSE: Less time will be devoted to the study of the toxication-detoxication mechanisms in small intestine and the influence of environmental pollutants on these systems in the next fiscal year. More emphasis will be placed on developing a program to study the influence of environmental pollutants on other biologic functions of intestine such as digestion, metabolism, and absorption of nutrients.

PUBLICATIONS

Tredger, J. M., Chhabra, R. S., and Fouts, J. R.: Postnatal development of mixed-function oxidation as measured in microsomes from the small intestine and liver of rabbits. Drug Metab. Disp. 4: 17-24, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80005-04 PB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

In Vitro Metabolism of Xenobiotics by Selected Marine Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Bend	Visiting Associate	PB	NIEHS
OTHER:	M. O. James	Visiting Associate	PB	NIEHS
	J. R. Fouts	Acting Scientific Director		NIEHS
	R. M. Philpot	Research Chemist	PB	NIEHS
	M. A. Q. Kahn	Visiting Scientist	PB	NIEHS
	R. J. Pohl	Research Biologist	PB	NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH

Pharmacology Branch

SECTION

Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the biotransformation of foreign organic compounds in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Both cytochrome P-450-dependent microsomal mixed-function oxidases and epoxide or arene oxide metabolizing enzymes (epoxide hydrase and glutathione S-transferases) are being characterized in control fish and in fish pre-exposed to environmental contaminants such as polycyclic aromatic hydrocarbons or dioxins.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: 1) To compare hepatic and extrahepatic microsomal xenobiotic oxidative metabolism in several vertebrate and invertebrate marine species, 2) to characterize the hepatic mixed-function oxidase system of the little skate, Raja erinacea, since it possesses relatively high enzyme activity, 3) to investigate some xenobiotic conjugating enzymes in marine vertebrates and invertebrates, and 4) to study the effects of environmental contaminant administration on in vitro xenobiotic mixed-function oxidation and conjugation by tissues from marine elasmobranchs and teleosts.

METHODS EMPLOYED: Subcellular fractions are prepared from tissue homogenates of freshly collected marine specimens. Standard procedures are utilized for the determination of enzyme activities and quantitation of microsomal cytochromes.

MAJOR FINDINGS: 1. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) (4.5 µg/kg on days 1 and 3) and DBA (1,2,3,4-dibenzanthracene) (10 mg/kg on days 1 and 3) pretreatment of little skates and winter flounder resulted in statistically significant increases (up to tenfold) in benzo[a]pyrene hydroxylase activity in microsomes prepared from liver. Hepatic microsomal cytochrome P-450 content was not altered by TCDD treatment of skates and there was no shift in the wavelength of the reduced P-450 complex with CO, similar to that seen in rats (from 450 nm to 448 nm). Similar responses were seen in skates after DBA administration. TCDD pretreatment did not affect microsomal epoxide hydrase or soluble fraction glutathione S-transferase activity in liver of little skate with styrene oxide as substrate.

2. Cytochrome P-450 was solubilized from little skate liver microsomes and purified 4- to 6-fold (over the microsomal concentration). Cytochrome b_5 was also separated from skate liver microsomes. The reductase has also been separated and the system is active in the metabolism of 7-ethoxycoumarin and benzphetamine when reconstituted.

3. Some properties of cytosol fraction glutathione S-transferase and microsomal epoxide hydrase activities were studied in liver and extrahepatic tissues of several marine species common to Maine or Florida using styrene oxide, octene oxide, or benzo[a]pyrene 4,5-oxide as substrate. Hepatic transferase activities were usually higher in teleost species than in those of elasmobranchs. Renal transferase activities were similar to hepatic values in elasmobranchs but were lower in kidney than in liver in teleosts.

Sheepshead (Archosargus probotocephalus) has typical hepatic glutathione transferase activities of about 20 nmoles/min/mg protein with styrene oxide as substrate and of approximately 50 nmoles/min/mg protein with benzo[a]pyrene 4,5-oxide as substrate. Transferase activity in cytosol fractions from intestine, gill, or spiral valve mucosa was much lower than in liver. Crustaceans have very high levels of epoxide hydrase in hepatopancreas microsomes (higher than rat microsomes). This is of interest since the same

microsomes have very low or undetectable microsomal mixed-function oxidase activity.

4. Hepatic microsomal mixed-function oxidase activities (with aniline, benzphetamine, benzo[a]pyrene, and aldrin as substrates) are being quantitated in the vertebrate and invertebrate marine species that are available at the Florida marine laboratory for comparison to similar data collected earlier at the Maine marine lab. Cytochrome P-450 levels are also being measured in the microsomal preparations and vary from 0.2-0.4 nmoles/mg protein for the sheepshead. Although hepatopancreas microsomes from the Florida lobster or crayfish (Panulirus argus) had undetectable benzo[a]pyrene hydroxylase activity; some of these preparations contained as much as 1 nmole cytochrome P-450/mg protein. However, individual variation was high and no cytochrome P-450 was detected in some of the preparations.

5. Hepatopancreas of the Florida lobster was shown to convert the cyclodiene insecticide aldrin to dieldrin. Highest specific activities for this mixed-function oxidase pathway were associated with the microsomal fraction, although significant activities were also present in mitochondrial and microsomal supernatant fractions.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The marine ecosystem is contaminated with substantial amounts of pesticides, industrial by-products and other synthetic organic chemicals which are produced in huge quantities by our technologically oriented society. The ability or inability of marine species, particularly those that are edible or those which are important in the aquatic food chain, to biotransform and excrete these xenobiotics is relevant both to the subsequent fate of these species (in the face of increasing pollution) and to their potential value and danger as direct and indirect foodstuffs for man.

PROPOSED COURSE; 1. Epoxide hydrase and GSH S-transferase activities towards epoxides and arene oxides of varying chemical reactivity will be studied to determine the substrate specificities of these two epoxide metabolizing enzymes in hepatic and extrahepatic tissues of several marine vertebrate and invertebrate species, both in control animals and in animals previously exposed to environmental pollutants. The profiles of GSH-transferases in liver supernatant (176,000 x g) fractions from selected Maine and Florida marine vertebrates (1 teleost and 1 elasmobranch) and invertebrates (lobster and crayfish) will be determined. Important transferases will be purified by conventional procedures and compared to their mammalian counterparts (specific activity, molecular weight, substrate specificities, isolectric points, inducibility, etc.). Hepatic microsomal epoxide hydrase will be purified from little skate liver microsomes from both control and polycyclic hydrocarbon induced animals.

2. With the reconstituted skate liver microsomal enzyme system (cytochrome P-450, lipid, and reductase fractions), we intend to identify the heat-sensitive fractions (relative to rabbit). This will be done by coupling a single skate component with the other two rabbit liver fractions and studying the effect of temperature increases on product formation.

3. The hepatic microsomal system of skates induced with a polycyclic hydrocarbon or TCDD (both of which we have shown to dramatically increase benzpyrene hydroxylase activities) will be solubilized, separated, and reconstituted. The cytochrome fractions will be compared with regard to substrate specificity, ability to form interaction spectra with type I and type II substrates, and spin state of the cytochrome.

4. The polycyclic hydrocarbon-induced hepatic microsomal mixed-function oxidase system will be characterized in some detail. Dose-response and time-response relationships will be defined.

5. In vitro mixed-function oxidation and conjugation rates for several xenobiotics will be measured in marine species acclimated to warm sea water in a Florida laboratory (water temperature ca. 27°C) for comparison to previous studies on Maine marine species acclimated to cold sea water (12-15°C). Studies with both control and MFO inducer-pretreated animals will be completed.

PUBLICATIONS

James, M. O., Fouts, J. R., and Bend, J. R.: In vitro epoxide metabolism in some marine species. Bull. Mt. Desert Island Lab 14: 41-46, 1975.

Bend, J. R., Peakall, D. B., Miller, D. S., and Kinter, W. B.: Effects of DDE administration on hepatic microsomal mixed-function oxidase activities in the puffin (Fratercula arctica) and the white Pekin duck (Anas platyrhynchos). Bull. Mt. Desert Island Biol Lab 14: 4-6, 1975.

Bend, J. R., Pohl, R. J., Davidson, N. P., and Fouts, J. R.: Response of hepatic and renal microsomal mixed-function oxidases in the little skate, Raja erinacea, to pretreatment with 3-methylcholanthrene or TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). Bull. Mt. Desert Island Biol. Lab 14: 7-12, 1975.

James, M. O., Pohl, R. J., Peret, D. G., Fouts, J. R., and Bend, J. R.: Further studies on epoxide metabolism in vitro by marine species. Bull. Mt. Desert Island Biol. Lab. In press, 1976.

Pohl, R. J., Fouts, J. R., and Bend, J. R.: Response of hepatic microsomal mixed-function oxidases in the little skate, Raja erinacea, and the winter flounder, Pseudopleuronectes americanus, to pretreatment with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) or DBA (1,2,3,4-dibenzanthracene). Bull. Mt. Desert Island Biol. Lab. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80006-05 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pollutants: Uptake, Distribution, Metabolism, Excretion, and Storage Sites In Marine Species

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Bend	Visiting Associate	PB	NIEHS
OTHER:	M. O. James	Visiting Associate	PB	NIEHS
	J. R. Fouts	Acting Scientific Director		NIEHS

COOPERATING UNITS (if any) Pharmacokinetics Section, Pharmacology Branch; Environmental Biometry Branch; and Laboratory of Toxicology, National Cancer Institute

LAB/BRANCH
Pharmacology Branch

SECTION
Marine Pharmacology and Biomedicine

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

The long range objectives of this project are to study the in vivo uptake, distribution, metabolism, and excretion of single, purified radiolabeled environmental contaminants, such as 2,4,5-trichlorophenoxyacetic acid, polychlorinated biphenyl isomers or hydrocarbons, in vertebrate and invertebrate marine species that serve as human food sources. The role of environmental temperature and exposure to other pollutants on the processes involved are also investigated.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this study are to measure the in vivo uptake, distribution, metabolism, and excretion of single, purified radiolabeled environmental contaminant, such as certain polychlorinated biphenyl (PCB) isomers or hydrocarbon isomers in selected marine species.

METHODS EMPLOYED: At various times after administration (generally parenteral) of radiolabeled compounds, specimens are sacrificed, plasma and tissues are removed, and aliquots are oxidized and/or solubilized prior to liquid scintillation counting. Urinary and biliary excretion rates are also monitored. Metabolites are identified by standard procedures, including glc, mass spectrometry, and NMR.

MAJOR FINDINGS: The herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) was excreted rapidly in the urine of female dogfish with cannulated urinary papillae. Smaller, but significant, amounts were excreted in the bile. A single, major metabolite was present in urine and bile, which was tentatively identified as 2,4,5-trichlorophenoxyacetyltaurine.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Since compounds such as 2,4,5-T, the PCBs, and some hydrocarbons are widespread environmental contaminants, the storage, metabolism, and elimination of these compounds in animals and man is of importance to environmental health. It is relevant to consider marine species since several pollutants are concentrated by the aquatic food chain and man relies upon several of these for a significant amount of foodstuff. Furthermore, the study of interaction between environmental contaminants, such as PCBs, and other xenobiotics including drugs, food additives, and pesticides with regard to competition for a common storage site in fish and other animals may aid in understanding increased toxicity of pollutant mixtures or increased storage of pollutants in fish exposed to pollutant mixtures.

PROPOSED COURSE: 1. The uptake, metabolism, and elimination of selected radiolabeled aliphatic and aromatic constituents of crude oil (petroleum) will be studied in at least one vertebrate and one invertebrate marine species acclimated to a cold water environment (Maine, 12-15°C) and to a warmer environment (Florida, 25-29°C). The effect of pre-exposure of experimental animals to foreign organic chemicals, such as the PCBs, chlorinated insecticides and hydrocarbons, and to heavy metals on the subsequent uptake, metabolism, and elimination of these aliphatic and aromatic radiolabeled crude oil constituents will also be studied in Maine and Florida marine animals.

2. Using xenobiotic conjugates which are excreted in urine or bile, such as ^{14}C-phenacetylglycine, the effect of environmental temperature on urinary and biliary excretion will be studied in Florida fish that have been acclimated to a variety of temperatures in the laboratory for a prolonged period (2 weeks-2 months) of time.

3. Skin of flounder and dogfish shark will be studied for their radioactive content following ^{14}C-2,4,5,2',5'-pentachlorobiphenyl administration, since rat skin serves as an important storage site for some polychlorinated biphenyl isomers.

PUBLICATIONS

Bend, J. R., Hart, L. G., Guarino, A. M., Rall, D. P., and Fouts, J. R.: Distribution and excretion of (^{14}C)-2,4,5,2',5'-pentachlorobiphenyl in the lobster (Homarus americanus) and the dogfish shark (Squalus acanthias). National Conference on Polychlorinated Biphenyls, Chicago, Illinois. In press, 1976.

James, M. O. and Bend, J. R.: Taurine conjugation of 2,4-dichlorophenoxyacetic acid and phenylacetic acid as a major metabolic pathway in two marine species. Xenobiotica. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80007-05 PB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Hepatic and Extrahepatic Conjugation and Oxidation Pathways in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Bend	Visiting Associate	PB	NIEHS
OTHER:	A. J. Ryan	Visiting Scientist	PB	NIEHS
	A. Aitio	Visiting Associate	PB	NIEHS
	Z. Ben-Zvi	Visiting Fellow	PB	NIEHS
	B. Smith	NIH Postdoctoral Fellow	PB	NIEHS
	R. M. Philpot	Research Chemist	PB	NIEHS

COOPERATING UNITS (if any) Environmental Toxicology Branch; Environmental Biometry Branch; Laboratory of Chemistry, NIAMDD; Laboratory of Biochemistry and Metabolism, NIAMDD

LAB/BRANCH

Pharmacology Branch

SECTION

Toxication-Detoxication

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.9	2.9	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

Several arene oxides and epoxides are known to react covalently with macromolecules, including nucleic acids, and to transform cells in vitro, suggesting their role as ultimate carcinogens, mutagens, and cytotoxins. We are studying the further metabolism of arene oxides and epoxides by soluble fraction glutathione S-transferases and microsomal epoxide hydrase in liver, lung, kidney, testis, and intestinal mucosa of adult and immature rats, rabbits, and guinea pigs. The relative importance of the two pathways for selected epoxides and arene oxides is being assessed in isolated perfused liver and lung, preparations which have intact cellular structures.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: 1) The characterization of some microsomal, mitochondrial or soluble conjugating and hydrating enzymes of extrahepatic tissues as compared to the liver enzymes; 2) to determine the effects of selected xenobiotics upon these enzymes; 3) to assess the relative importance in vivo of this extrahepatic enzyme activity as compared to the liver following ingestion or administration of chemicals and drugs (substrates) via various routes including inhalation; 4) to investigate the perinatal development of some detoxifying enzymes in hepatic and extrahepatic tissues of a few species and to determine the protective role, if any, of these enzymes against localized tissue toxicity induced by chemically reactive electrophilic metabolites, such as epoxides; and 5) to compare the metabolism of chemically reactive epoxides and their unsaturated hydrocarbon precursors in isolated perfused rabbit lung and rat liver preparations.

METHODS EMPLOYED: Differential centrifugation, spectroscopic, fluorometric and radiochemical analyses, and organ perfusion procedures.

MAJOR FINDINGS: 1. In isolated perfused rat liver, 27-40% of an administered dose of ^{14}C-styrene oxide (SO) was excreted in the bile during a 90-minute period with maximum biliary excretion occurring between 20 and 30 minutes after addition of SO to the perfusate. Reverse isotope dilution analysis demonstrated that S-(2-hydroxy-1-phenyl) ethyl glutathione was the only major biliary metabolite. Styrene glycol, mandelic acid and the GSH derivative were the major metabolites present in the perfusate, being present in approximately equal concentrations. There was negligible covalent binding of the radioactivity associated with ^{14}C-styrene oxide to liver proteins.

2. Rat liver 176,000 x g supernatant was shown to have glutathione S-transferase activity towards a number of arene oxides and epoxides including octene oxide, styrene oxide, benzo[a]pyrene 4,5-oxide, benzo[a]pyrene 7,8-oxide, benzo[a]pyrene 9,10-oxide, benzo[a]pyrene 11,12-oxide, benzanthracene 5,6-oxide, dibenz[a,h]anthracene 5,6-oxide, phenanthrene 9,10-oxide, and 3-methylcholanthrene 11,12-oxide. Specific activity of the enzyme showed substrate variation with the different oxides. The 176,000xg supernatant fractions from rat lung and small intestine had 6-25% of the specific activity for the equivalent fraction from liver with each epoxide studied, while the fractions from kidney and testes had 20-100% of liver specific activity. Epoxide concentration-activity, GSH concentration-activity, pH-activity, incubation temperature-activity and protein concentration-activity relationships were determined for styrene oxide, octene oxide, and benzo[a]pyrene 4,5-oxide with rat liver cytosol. 5 μM benzo[a]-pyrene 4,5-oxide was found to inhibit glutathione S-transferase activity towards styrene oxide by 50%. Conversely, cyclohexene oxide (10 mM) had no effect on the enzymatic formation of the glutathione derivative of styrene oxide.

3. The metabolism of benzo[a]pyrene has been compared in isolated perfused lungs from control rabbits and from rabbits pretreated with 3-methylcholanthrene (3-MC). The compound was administered to the lung via the perfusion medium.

Metabolites were quantified by group, using TLC analysis. 3-MC pretreatment did not significantly affect the rate of metabolism of benzo[a]pyrene in lung or the covalent binding of the radioactivity associated with this hydrocarbon to rabbit lung. 3-MC also did not alter benzo[a]pyrene uptake from the perfusion medium.

4. About 10% of the benzo[a]pyrene 4,5-oxide (2 μmoles) added to the perfusate (150 ml) circulating through isolated perfused rat liver was excreted in the bile over a 90-minute period--primarily as a single metabolite with the chromatographic properties of a glutathione conjugate. A considerable amount of the radioactivity was tightly bound to liver tissue.

5. Apparent Vmax and Km values have been determined for a number of epoxide and arene oxide substrates (both K- and non-K-regions) with purified rat liver transferases B and C. Substrates studied included benzo[a]pyrene 4,5-, 7,8-, 9,10-, and 11,12-oxides, dibenz[a,h]anthracene 5,6-oxide, benzo[a]-anthracene 5,6-oxide, 3-methylcholanthrene 11,12-oxide, phenanthrene 9,10-oxide, styrene oxide and octene oxide. Apparent Km values for the arene oxides are in the μMolar concentration range (1.4-14.5 μM) for both enzymes whereas they are in the mMmolar range for the epoxides studied (0.5-4.4 mM).

6. The activity of a purified human glutathione S-transferase with the various arene oxides as substrate has also been determined. Rates varied from 0.2 nmole/min/mg protein for benzo[a]pyrene 7,8-oxide to 22 nmoles/min/mg protein for benzo[a]pyrene 4,5-oxide. All arene oxides tested were substrates for this enzyme.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The conjugation of xenobiotics or their metabolites which are chemically reactive (e.g., epoxides and arene oxides) by liver, lung, gut, and other extrahepatic tissues may play a significant role in the overall toxicity of agents which enter the body via the pulmonary system or the alimentary canal. The developmental patterns of conjugation pathways active in detoxication are of importance since these may determine the susceptibility of the fetus and neonate to environmental contaminants and may also be related to chemical carcinogenesis and teratogenesis. In the real-life situation one is very rarely exposed to a single xenobiotic; rather, one comes in contact with a large number of synthetic organic chemicals including food additives, drugs, plasticizers, insecticides, hydrocarbons, etc. For this reason investigations concerning interactions between more than one foreign compound are of relevance to environmental health.

PROPOSED COURSE: 1. The metabolism of styrene will be studied in isolated perfused rat liver and rabbit lung preparations for comparison to earlier investigations made with styrene oxide. The disposition of other epoxides and arene oxides and their hydrocarbon precursors will also be determined. Livers from control, induced, and glutathione-depleted rats will be tested and acute toxicity will be assessed by tight (covalent) binding of radioactivity to

protein.

2. Using a purified rat liver glutathione S-transferase, inhibition experiments will be conducted using a few epoxides and arene oxides as substrates and a number of chemically divergent chemicals, including epoxides and arene oxides, as inhibitors. The type of inhibition will be determined in each instance.

3. Glutathione S-transferase profiles will be determined for liver of fetal and neonatal rats for comparison to adult animals. If sufficient purified enzyme can be obtained from the immature animals the properties of the enzymes will be compared with enzymes from adults. Similar studies are contemplated with extrahepatic tissues.

PUBLICATIONS

Bend, J. R., James, M. O., Devereux, T. R., and Fouts, J. R.: Toxication-Detoxication systems in hepatic and Extrahepatic Tissues in the Perinatal Period. In: Proceedings of the International Symposium on Perinatal Pharmacology. New York, Raven Press, 1975, pp. 229-243.

Bend, J. R., Ben-Zvi, Z., Van Anda, J., Dansette, P. M., and Jerina, D. M.: Hepatic and Extrahepatic Glutathione S-Transferase Activity Towards Several Arene Oxides and Epoxides in the Rat. In Freudenthal, R. I. and Jones, P. W.: Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis. New York, Raven Press, 1976, pp. 63-75.

Jerina, D. M. and Bend, J. R.: Glutathione S-Transferases. In: Proceedings of Symposium on Active Metabolites: Formation, Toxicity and Inactivation held July 26-27, 1975, Turku, Finland. In press, 1976.

Ryan, A. J., James, M. O., Ben-Zvi, Z., Law, F. C. P., and Bend, J. R.: Hepatic and extrahepatic metabolism of 8-^{14}C-styrene oxide. Environ. Hlth. Persp. In press, 1976.

Hook, G. E. R. and Bend, J. R.: Pulmonary metabolism of xenobiotics. Life Sciences 18: 279-290, 1976.

James, M. O., Fouts, J. R., and Bend, J. R.: Hepatic and extrahepatic in vitro metabolism of an epoxide (8-^{14}C-styrene oxide) in the rabbit. Biochem. Pharmacol. 25: 187-193, 1976.

Harper, C., James, M. O., Devereux, T. R., Patel, J. M., Bend, J. R., and Fouts, J. R.: Characteristics and development of drug metabolism by pulmonary microsomes. Agents and Actions. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80008-02 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Transport and Metabolism of Prostaglandins [PG] in Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Research Chemist	PB	NIEHS
	M. W. Anderson	Senior Staff Fellow	EBB	NIEHS
OTHER:	A. G. E. Wilson	Visiting Fellow	PB	NIEHS

COOPERATING UNITS (if any)

Inhalation Toxicology Section, Environmental Biology and Chemistry Branch

LAB/BRANCH
Pharmacology Branch, Environmental Biometry Branch

SECTION
Pharmacokinetics Section

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.4	1.3

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range goal of this project to study the inactivation of prostaglandins by the lungs and other tissues. The effects of environmental agents on the pulmonary transport and metabolism of prostaglandins is being investigated, and these effects are being related to pulmonary toxicity and damage caused by the environmental agents. Topics of current interest are the structural requirements of a prostaglandin needed for transport to intracellular spaces. The isolated perfused lung preparation is being used extensively in these studies.

PROJECT DESCRIPTION

OBJECTIVES: 1) To elucidate the mechanism(s) for the transport of prostaglandins (PGs) across cell membranes, 2) to determine the effects on this transport system of various anti-inflammatory agents and exposures of animals to pollutants, and 3) to relate alteration in the PG-transport system to physiological effects of pollutants on the lung.

METHODS EMPLOYED: An isolated perfused rat lung was used to examine the uptake, metabolism, and efflux of PGs and their metabolites from lung tissue. The isolated perfused lung was designed to permit infusion of a constant concentration of PGs and perfusion with drug-free perfusate. PG metabolites were isolated from the perfusate by extraction and separated by TLC techniques. The unidirectional flux of PG into the lung was measured by extrapolation of the net uptake velocity of PG to zero time.

MAJOR FINDINGS: ^{3}H-$PGF_{2\alpha}$ and ^{3}H-PGE_1 accumulated into lung tissue by a carrier-mediated process. Accumulated PG was metabolized to either 15-keto-PG_{α} or 15-keto-dihydro PG_{α}. The accumulation of PG_{α} and the subsequent efflux of PG_{α} and its metabolites were very rapid. The transport process for PGs in rat lung was specific; $PGF_{2\alpha}$ and PGE_1 were accumulated in lung while PGB_1 and PGA_1 were not accumulated in lung tissue. The metabolite 15-keto-$PGF_{2\alpha}$ was not taken up by the lung but when formed inside the cell from $PGF_{2\alpha}$ it was rapidly effluxed from the lung. This indicates that lung cell membranes may be asymmetric with respect to PG passage. The transport of PGs appears to be related to the rate at which PGs are supplied to the lung rather than to the concentration in the perfusate. Supply rate (perfusate concentration x flow rate) should be used in species-to-species comparisons of transport systems in isolated organ systems.

PGE_2 was found to competitively inhibit the uptake of PGE_1 into the rat lung which is consistent with a carrier-mediated transport system for the removal of PG from the circulation. The non-steroidal anti-inflammatory drug, indomethacin, did not affect the uptake of PG by the lung, however, the steroid anti-inflammatory agents, hydrocortisone and dexamethasone, appear to inhibit PG transport. This inhibition by steroids could be related to their anti-inflammatory action. The PG antagonist SC-19222 and 7-oxo-prostanoic acid did not inhibit PG removal by the lung while the antagonists PPP and DPP were potent inhibitors.

Red blood cells were also examined as a potential model for studying the PG transport system in more detail. Rat red blood cells were found to exclude PGE_1 and $PGF_{2\alpha}$ and not metabolize these PG. PGA_1 was degraded by rat red blood cells but no accumulation into these cells was observed. PGA_1 appears to be metabolized by enzymes located either in the plasma or red blood cell membranes. This supports our hypothesis -- no PG transport for PGA_1 exists in red blood cells or in guinea pig lung. Reported PGA_1 transport is erroneous. This hypothesis is currently being investigated

further. Interestingly, human red blood cells do contain PG dehydrogenase enzymes and would have a PG transport system. Transport of PG in human red blood cells will be investigated.

Structural requirements for PG transport in the lung are being investigated. Present evidence indicates that the distance between the oxygen functions at positions 9 and 15 is critical. PGE_1, $PGF_{2\alpha}$, PGE_2, $PGF_{1\alpha}$, and PGD_2 are transported while PGA_1, PGB_1, 15-keto-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-PGE_1, and 13,14-dihydro-PGE_1 are not transported. Other PG isomers are currently being studied to determine essential structural features required for transport.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: PGs have a large diversity of physiological effects. Alterations in PG control of cellular events may be related to transport of PGs across cell membranes. The lung is an important site for the synthesis and metabolism of PGs; alterations in the PG transport and metabolism systems may be related to toxic effects of exposures to pollutants and induction of lung diseases.

PROPOSED COURSE: 1) To further study the effect of anti-inflammatory drugs on PG transport in the lung, 2) to examine the effect of exposure of rats to airborne pollutants on PG detoxification systems, 3) to use isolated cell systems to examine the mechanisms for the transport of PG into and out of cells and apply the information obtained from this study to the intact lung system, 4) to relate PG transport in cells to cellular levels of cyclic AMP-GMP and cell proliferation, 5) to examine PG transport in human red blood cells, and 6) to further study the structural requirements for PG transport.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 ES 80009-05 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pharmacokinetic Functions of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Research Chemist	PB	NIEHS
	M. W. Anderson	Senior Staff Fellow	EBB	NIEHS
OTHER:	A.G.E. Wilson	Visiting Fellow	EBB	NIEHS

COOPERATING UNITS (if any)

Inhalation Toxicology Section, Environmental Biology and Chemistry Branch

LAB/BRANCH
Pharmacology Branch, Environmental Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to examine pharmacokinetic functions of the lung with regard to both endogenous and exogenous chemicals. The pharmacokinetics of these chemicals will be related to environmentally induced pulmonary damage. An isolated perfused lung is used which permits examination of the pharmacokinetics of chemicals reaching the lungs both from the circulatory system and from the inhaled air.

PHS-6040
(12-75)

PROJECT DESCRIPTION

PART I: "Studies on the Mechanisms of Accumulation and Metabolism of Chemicals and Pollutants by the Lung"

OBJECTIVES: Study the processes and mechanisms for uptake and accumulation of chemicals in lung tissue. Assess the role of the lung in the disposition of chemicals and develop pharmacokinetic models for disposition of chemicals in the lung.

METHODS EMPLOYED: The lung perfusion apparatus consists of an artificial thorax, a recirculating blood supply, and an air supply. The lungs are ventilated with 95% air - 5% CO_2 by applying an alternating negative pressure to the artificial thorax. The circulatory system was designed with a blood reservoir directly above the lungs to which the labeled chemicals were added and from which samples of blood were removed for analysis. The apparatus was modified so that the rates of uptake and the rates of efflux of chemicals from lung could be examined.

Metabolites of the compounds as found in the blood and lung were studied by thin-layer, paper, and gas liquid chromatographic techniques. Viability of the perfused lung was confirmed by physiologic parameters as well as by light and electron microscopy.

The perfused lung is designed to allow an examination of the metabolism and retention of inhaled chemicals. The trachea is connected directly to an aerosol generator and the appearance of the chemical and its metabolites in the circulating media is monitored.

MAJOR FINDINGS: We have developed detailed models for the dynamic interaction of the perfused lung and circulating basic amines including amphetamine (AMPH), imipramine (IMP), and methadone (MET). All of these compounds rapidly diffuse into and out of the lung's vascular space. Little AMPH was bound in the intracellular space while significant amounts of IMP and MET partitioned to the intracellular space. AMPH was accumulated into the cells by a carrier-mediated transport process. IMP and MET were accumulated by processes of diffusion and binding. Approximately 10-15% of accumulated MET was metabolized by the MFO system. AMPH did not persist in the lung and effluxed very rapidly from the tissue once influx of AMPH stopped. IMP and MET effluxed with a half-life of 8 minutes. Approximately 20-30% of accumulated IMP and MET was not effluxable. This non-effluxable pool is responsible for the persistence of basic amines in lung tissue which occurs after blood levels of the chemical become zero. The non-effluxable pools for IMP and MET were not the result of covalent binding of IMP and MET but appeared to be the result of compartmentalization of these chemicals. The non-effluxable pool for certain amines may be associated with phospholipid-chemical complexes in concentric lamellar organelles (CLO) and may be involved in pulmonary phospholipidosis produced by a variety of basic amines (e.g., chlorphentermine).

In addition, in vivo studies with IMP have shown that the non-effluxable pool in lung does form in the intact rabbit and has a half-life of 6 hours. More than 90% of the drug remaining in the lung 8 hours after an administration of IMP was associated with this pool. Studies with isolated perfused rat lung indicate that IMP does form a non-effluxable pool in rat but of much smaller size. The difference in the size of the non-effluxable pool between rabbit and rat correlated with the difference in the phospholipid to protein ratio in lung concentric lamellar organelles (CLO) (rabbit 10:1; rat 4:1).

Other studies deal with the uptake and persistence of paraquat and diquat in the isolated perfused rabbit lung. Differences in the amount and persistence of paraquat and diquat in lung may correlate with the difference in the pulmonary toxicity of these two herbicides. Studies on the uptake and persistence of paraquat are underway in rat in an attempt to examine increased toxicity of paraquat in rat compared to rabbit on the basis of high levels of paraquat in rat lung.

We have also studied the metabolism of several chemicals by the isolated perfused rabbit lung. Of the chemicals studied, only parathion, methadone, and pentobarbital were significantly degraded by isolated perfused rabbit lung. However, parathion, methadone, pentobarbital, imipramine, amphetamine, and chlorcyclizine were all degraded by lung and liver homogenates and subcellular fractions. Methadone was metabolized to two cyclic metabolites (M_1, M_2) by both lung homogenates and the isolated perfused lung. However, the ratio of M_1 to M_2 obtained with lung homogenates was different from that obtained in isolated perfused lung. Thus, extrapolation of metabolism data from in vitro incubation mixtures to intact lung may not always be possible.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Development of a method for the perfusion of an isolated intact lung allows studies with the lung uncomplicated by other organ functions. This preparation is perfused and ventilated similarly to an intact organ and thus good correlation of in vivo and in vitro studies is likely.

The lung can act as an important site for accumulation of xenobiotics and this accumulation could result in detoxification or altered toxicity. In addition, those xenobiotics which accumulate in the lung could affect the accumulation and storage of other exogenous compounds or of endogenous substances such as norepinephrine and 5-hydroxytryptamine. The elucidation of the mechanism of accumulation could afford a means for predicting possible toxic effects and interactions of one chemical with another.

Examination of the non-effluxable pool can permit development of methods for predicting or modifying chemical-induced lipidoses in lung tissue.

PROPOSED COURSE: 1) To examine the efflux characteristics of phentermine and chlorphentermine in the isolated perfused rabbit lung and to relate the presence of a non-effluxable pool of a chemical or drug to the production of lung phospholipidosis by these chemicals, 2) to study the interaction

between endogenous and exogenous amines at their sites of lung uptake and release, 3) to develop pharmacokinetic models for the accumulation of amines in lung tissue, 4) to assess the role of lung phospholipid and CLO in the accumulation and persistence of basic amines, 5) further study of paraquat uptake and persistence in lung and mechanism of pulmonary toxicity, and 6) to examine the uptake and metabolism of inhaled chemicals.

PART II: "Studies on the Uptake and Metabolism of Endogenous Substances by the Lung"

OBJECTIVES: To study the uptake and metabolism of bioactive endogenous substances, such as 5-hydroxytryptamine, norepinephrine, prostaglandins, and angiotensin by lung. Examine the interactions between the accumulation of endogenous and exogenous substances by lung and study the effect of inhaled environmental agents on a such non-respiratory function of the lung.

METHODS EMPLOYED: See "Methods Employed" in Part I.

MAJOR FINDINGS: Serotonin (5-HT) is removed from the venous circulation in lung by a unidirectional transport system which is Na^+ dependent. 5-HT is metabolized by the intracellular monoamine oxidase to 5-hydroxyindole acetic acid and this metabolite escapes by diffusion back into the arterial circulation. We have shown that the components of this detoxification system for 5-HT can be separately inhibited. For example, we have inhibited the metabolism step without affecting the transport system by using the monoamine oxidase inhibitor, pargyline. The 5-HT which was transported into the cell returned unaltered to the circulation by diffusion. Conversely, imipramine inhibits the transport system without affecting the metabolism.

A pharmacokinetic model was developed for the 5-HT detoxification system. From this model it was evident that flow rates are important in examining transport systems in isolated organs. Thus, development of a pharmacokinetic model has identified potential erroneous experimental designs in studying transport systems in perfused organs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The lung is an important site for the detoxification of several endogenous compounds. Understanding the mechanism(s) for these detoxifications, etc., could aid in understanding lung function and the effect of certain environmental insults on the lung.

Elucidation of the mechanism of lung uptake and metabolism of chemicals might allow prediction of possible toxic interactions between endogenous and exogenous substances.

PROPOSED COURSE: We will examine the effect of exposure to pollutants on the 5-HT detoxification system of the lung.

PUBLICATIONS

Pickett, R. P., Anderson, M. W., Orton, T. C., and Eling, T. E.: The pharmacodynamics of 5-hydroxytryptamine uptake and metabolism by isolated perfused rabbit lung. J. Pharmacol. Exp. Ther. 194: 545-553, 1975.

Eling, T. E., Pickett, R. P., Orton, T. C., and Anderson, M. W.: A study of the dynamics of imipramine accumulation in the isolated perfused rabbit lung. Drug Metab. Disp. 3: 389-399, 1975.

Anderson, M. W., Eling, T. E., and Philpot, R. M.: Metabolic functions of lung. In Lenfant, C. (Ed.): Monograph 5 of the series entitled "Lung Biology in Health and Diseases." Uptake, Disposition, and Metabolism of Chemicals by the Lung. Chapter 5. Marcel Dekker, Inc., New York. In press.

Law, F. C. P., Wilson, A. G. E., Eling, T. E., and Anderson, M. W.: Uptake, metabolism, and efflux of methadone in "single pass" isolated perfused rabbit lungs. J. Pharmacol. Exp. Ther. In press, 1976.

Anderson, M. W. and Eling, T. E.: The use of isolated perfused lung to examine the uptake and metabolism of chemicals. Environ. Hlth. Persp. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80010-05 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Prostaglandin Biosynthesis and Metabolism by Lungs. Effect of Pollutants on this System.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Eling	Research Chemist	PB	NIEHS
	M. W. Anderson	Senior Staff Fellow	EBB	NIEHS
OTHER:	A. Chaudhari	Visiting Fellow	PB	NIEHS
	J. R. Hass	Staff Fellow	EBCB	NIEHS
	W. Lynn	Professor, Dept. of Medicine	Duke University	

COOPERATING UNITS (if any)
Inhalation Toxicology Section, Environmental Biology and Chemistry Branch; Duke University

LAB/BRANCH
Pharmacology Branch, Environmental Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.9	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to study the biosynthesis, release, and metabolism of prostaglandins (PG) by pulmonary tissue and to relate altered PG biosynthesis, release, or metabolism to pulmonary diseases. Also of interest is to study prostaglandins as early indicators of pulmonary damage induced by exposure to environmental agents. Emphasis is being placed on studying the biochemical mechanisms that control the release of PG precursors, unsaturated fatty acids, from lung phospholipids and, thus, partially controlling PG biosynthesis.

PROJECT DESCRIPTION

OBJECTIVES: This is a continuing project directed toward obtaining a better understanding of lung function. The primary objectives are to investigate the biosynthesis, release, and metabolism of prostaglandins by the lung and to relate changes in steady-state concentrations of the various prostaglandins to biological effects. In addition, the effects of various environmental agents (SO_2, O_3, NO_2, O_2, etc.) on the ability of the lung to synthesize, release, and degrade prostaglandins (PG) will be investigated. Attempts will be made to correlate the biological effect of pollutants and modulation of PG levels with human pulmonary diseases. Altered PG biosynthesis and release in pathologic states will be examined as potential markers for pulmonary damage.

METHODS EMPLOYED: Prostaglandins are analyzed by a combination of quantitative thin-layer chromatography and liquid scintillation counting. The radioactive PG produced by lung tissue from the precursor ^{14}C-arachidonic acid are extracted from the incubation medium and separated on thin-layer plates. The radioactive areas are measured by liquid scintillation techniques. Guinea pig or rat lung serves as an enzyme and co-factor source for both biosynthesis and metabolism. Further identification of PG uses a combination of thin-layer chromatography, continuous gradient elution column chromatography and mass spectrometry. Prostaglandin metabolism was estimated by incubation of ^{3}H-$PGF_{2\alpha}$ with the enzyme source, extraction from the incubation mixtures, and separation of nonmetabolized ^{3}H-$PGF_{2\alpha}$ from metabolites. Metabolism was measured by the disappearance of ^{3}H-$PGF_{2\alpha}$. The isolated perfused lung is used to study release of PG from the lung as a result of environmental insults.

MAJOR FINDINGS: Lung tissue biosynthesizes and degrades PG. The PG synthesizing system in guinea pig lung converts ^{14}C-arachidonic acid to $PGF_{2\alpha}$ and an unknown PG-like material. An assay for the enzyme system was developed, characterized, and optimized. We have established the chemical identity of $PGF_{2\alpha}$ produced by our enzyme system with GLC-mass spectrometry. The unknown has been identified by mass spectrometric techniques as 8-(1-hydroxy-3-oxopropyl)-9,12-dihydroxy-5,10-heptadecadienoic acid. This compound has recently been isolated from guinea pig lung incubation mixtures and designated PHD. From our studies and those of other workers, it is apparent that guinea pig lung does not biosynthesize large amounts of PGE_2 and $PGF_{2\alpha}$ but produces large amounts of other "prostaglandin-like" compounds. These "prostaglandin-like" compounds are produced by conversion of an unstable bioactive cyclic intermediate in PG biosynthesis. The bioactivities of PHD and the other products from the cyclic intermediate have not been assessed.

PGs are metabolized by guinea pig and rat lung dehydrogenases and reductases. Using ^{3}H-$PGF_{2\alpha}$ as substrate, an in vitro assay system was developed and the metabolites isolated and identified by combined GLC-mass spectrometry.

The effect of environmental agents on the rates of biosynthesis and metabolism in vitro were investigated. We have established that exposure of guinea pigs to SO_2 at various concentrations did not alter PG biosynthesis and metabolism. However, exposure of guinea pigs to 100% O_2 depressed PG metabolism but did not alter biosynthesis. Preliminary experiments indicated that exposure of rats to H_2SO_4 mist inhibited PG metabolism while exposure of rats to O_3 did not alter PG degradation. We are currently investigating these effects in more detail.

Exposure of animals to 100% O_2 was used as a model for exposure to other oxidant gases. Exposure of guinea pigs to 100% O_2 depressed the rate of PG metabolism by the enzyme 15-prostaglandin dehydrogenase in lung, but metabolism of PG in the kidney and spleen by this enzyme was not inhibited by O_2 exposure. Exposure of rats to 100% O_2 also inhibited PG metabolism by the lung. Thus, the toxicity indicated by depression of PG metabolism is not species-dependent but is specific to the lung. $NADP^+$-dependent PG metabolism in lung of exposed guinea pig was also inhibited. We have studied several additional dehydrogenase enzymes in lung but no effect of O_2 exposure was found. Depression of PG metabolism may be associated with the decrease of pulmonary Type I cells resulting from the exposure of animals to oxidant gases. Thus, measurement of PG metabolism may serve as an indicator of lung damage. The relationship of PG metabolism to the alveolar Type I cell is under investigation in the hope of developing PG metabolism as an early warning system for pulmonary damage.

The lung has been found to produce, from arachidonic acid, PGE_2, $PGF_{2\alpha}$, thromboxane A_2 and B_2, HETE and HTT. We have found that guinea pig lung produces greater amounts of thromboxanes from PGE_2 and $PGF_{2\alpha}$. This is of importance since thromboxanes have been shown to be approximately 1000 times more biologically active than PGE_2 or $PGF_{2\alpha}$. In addition, guinea pig lung does produce some HETE which has been shown to chemotactic. We have found that rat lung produces small amounts of PG and PG-like substances and equal amounts of thromboxane B_2, $PGF_{2\alpha}$, and PGE_2. No information is available on PG synthesis in other species and in man. Cells obtained in alveolar washing (PMN, macrophages, etc.) also synthesize PG but the kinds and amount of the PG have not been examined.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
1. PG and PG-like substances represent a class of endogenous substances which possess unique biological activity. PGs have been implicated in inflammation and control of smooth muscle tone. The isolation and purification of the cyclic intermediates and the thromboxanes opened a whole new vista of biological effects related to the PGs. Cyclic intermediates and thromboxanes have very potent effects on smooth muscle, lung airways, platelets and adenyl cyclase. Because PG and PG-like substances are not stored in tissues, control of biosynthesis (release of arachidonic acid and conversion to PG and PG-like substances) and metabolism is most important to understanding their physiological activity.

Pathological changes or conditions produced in various disease states or in response to pollutants or environmental agents could involve an effect on either prostaglandin biosynthesis or metabolism. Understanding the mechanisms for the control of prostaglandin levels in lung tissues should contribute to the overall understanding of biological functions of these lipids.

2. This project will be done in collaboration with the pulmonary research group at Duke University. These studies represent a unique application of basic research to human pulmonary diseases. The investigations will permit the Institute to examine human pulmonary disease induced by environmental agents. Determination of the role of prostaglandins in lung function and in pulmonary diseases, particularly diseases which are environmentally related, are our basic objectives.

PROPOSED COURSE: 1. Study the effect of oxidant gases on PG metabolism and relate inhibition of PG metabolism to decreased numbers of Type I cells in lung. Development as an indicator for early damage to the lung.

2. Examine the effect of other oxidant gases and environmental pollutants on lung PG metabolism.

3. Examination of PG synthesis in the lung of rat, rabbit, dog, and monkey. Human lung samples will be obtained from Duke University.

4. Study the release of PG from the lung as a result of environmental insults. Little information is available on the release of PG from the lung into the circulation and alveolar spaces as a result of various stimuli.

5. Measure the levels of PG and PG-like materials in the alveolar washings from human patients with pulmonary disease and relate PG to these diseases.

6. Study PG systems in the cellular components obtained from humans suffering environmentally induced lung diseases.

7. Study the mechanism responsible for the release of PG precursor, arachidonic acid, from lung phospholipid. Examine the various cellular preparations for PG synthetase activity and specific phospholipases. Washings from patient with bronchial asthma will be studied for possible increased or altered phospholipase activity.

PUBLICATIONS

Eling, T. E. and Anderson, M. W.: A study of biosynthesis, metabolism and transport in lung. Agents and Actions. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80012-02 PB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Disposition of Polychlorinated Biphenyls by the Isolated Perfused Liver

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. M. Mehendale	Staff Fellow	PB	NIEHS
OTHER:	H. B. Matthews	Research Chemist	PB	NIEHS
	T. E. Eling	Research Chemist	PB	NIEHS
	M. W. Anderson	Senior Staff Fellow	EBB	NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH

Pharmacology Branch

SECTION

Pharmacokinetics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The isolated perfused rat liver system (IPL) was used to study the kinetics of uptake and excretion for four polychlorinated biphenyl isomers (PCBs). All four were rapidly taken up from the perfusate by the liver, and the rate of biliary excretion was greatest with the least chlorinated isomer (4-chlorobiphenyl) and slowest with the most chlorinated isomer (2,4,5,2',4',5'-hexachlorobiphenyl). The rate of biliary excretion for the two less chlorinated isomers was exponentially related to their hepatic concentration, but for the two more highly chlorinated isomers excretion was not related to hepatic concentration.

PROJECT DESCRIPTION

OBJECTIVES: We developed an isolated perfused rat liver preparation (IPL) suitable for pharmacokinetic studies with xenobiotics. This IPL was used to study uptake, metabolism, accumulation, and excretion of PCBs. We used a homologous series of PCBs (4-chlorobiphenyl, I; 4,4'-dichlorobiphenyl, II; 2,4,5,2',5'-pentachlorobiphenyl, III; and 2,4,5,2',4',5'-hexachlorobiphenyl, IV) to investigate the relationship of degree of chlorination of PCBs to their uptake, metabolism, and excretion by the liver preparations. These studies were directed towards determining the role of the liver in the overall whole animal disposition of PCBs.

METHODS EMPLOYED: The liver perfusion apparatus developed for this work was a modified version of the Miller apparatus. Rat liver was surgically removed after cannulating the bile duct, portal vein, and the thoracic vena cava. The inferior vena cava was ligated. The liver was perfused with 30% rat blood in Krebs-Ringer buffer solution (pH 7.4) containing 5 mM bovine serum albumin and 4.5 mM glucose. Oxygenated blood entered through the portal vein and effluxed via the thoracic vena cava cannula. These experiments were conducted using a recirculating perfusate. Labeled PCBs were introduced into the perfusate at zero time after the liver had equilibrated for 30 min. Bile, perfusate, and liver homogenate samples were assayed for radioactivity. Liver homogenate, perfusate, and bile samples were also extracted and the total radioactivity divided into parent compound and metabolites using conventional TLC methods.

MAJOR FINDINGS: An isolated perfused rat liver preparation suitable for pharmacokinetic studies was developed and is being used to study the disposition of PCB homologs by the rat liver. Viability of the preparation was ascertained by a combination of tests including visual examination, glucose and oxygen utilization by the liver, and bile flow as well as histological examination of the liver after varying periods of perfusion up to 5 hours. All four PCB homologs were rapidly taken up by the liver from a recirculating perfusate, and after the initial 30 minutes a steady-state was established with respect to the total radioactivity in the perfusate. The rate of biliary excretion was greatest with compound I, followed by II, III, and IV, respectively, indicating an inverse relationship with the degree of chlorination. Biliary excretion and hepatic uptake were linear for 1, 3, and 10 nmole PCB/ml perfusate of compounds I and IV. The rate of biliary excretion of compound I was exponentially related to its concentration in the liver. A similar relationship existed for compound II. However, biliary excretion of compounds III and IV was not related to hepatic concentration. The concentration of unchanged I in the perfusate declined biexponentially corresponding to a 2-compartment model: the initial rapid phase of uptake by the liver ($t_{\frac{1}{2}}$ = 7 min) was followed by a second slow phase of uptake ($t_{\frac{1}{2}}$ = 270 min).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Due to the widespread occurrence of PCBs in the environment and in all the biological species examined including man, there is increasing concern about the effects of these chlorinated compounds on human health. These studies are a part of efforts to construct a pharmacokinetic model for the uptake and disposition of PCBs in animals. This particular study is directed towards understanding and quantifying the role of the liver in the whole body disposition of PCBs.

PROPOSED COURSE: This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80014-03 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Metabolism of Polychlorinated Biphenyls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Matthews	Research Chemist	PB	NIEHS
	L. T. Jao	Visiting Fellow	PB	NIEHS
	J. R. Hass	Staff Fellow	EBCB	NIEHS

COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch

LAB/BRANCH
Pharmacology Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

SUMMARY OF WORK (200 words or less - underline keywords)

Metabolism to more polar compounds is a prerequisite to polychlorinated biphenyl (PCB) excretion by animals. Two adjacent unsubstituted carbon atoms are necessary for an appreciable rate of metabolism of the PCBs. The major metabolites of PCB are mono- and dihydroxylated derivatives which are usually excreted as glucuronide conjugates. There is good evidence to suggest that the PCBs are metabolized by hepatic mixed-function oxidases to their respective hydroxylated derivatives via arene oxide intermediates.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: To determine the importance of position and degree of chlorination of the PCBs on their rate of metabolism and the nature of the metabolites formed.

METHODS EMPLOYED: The studies have utilized ^{14}C-labeled PCB isomers and in vivo metabolism of these isomers by the rat to obtain the necessary metabolites. The metabolites were isolated by various extraction techniques, purified by thin-layer and column chromatography, and identified by mass spectral and nuclear magnetic resonance analysis.

MAJOR FINDINGS: 1. The major metabolites of the PCBs are mono- and dihydroxylated PCBs and there is a possibility of an epoxide intermediate. Metabolism appears to be a prerequisite to PCB excretion. Two adjacent unsubstituted carbon atoms are necessary for an appreciable rate of metabolism of the PCBs.

2. The major metabolites of 4-chlorobiphenyl have been isolated and identified as: 4-hydroxy-4'-chlorobiphenyl; 3-hydroxy-4'-chlorobiphenyl; 2,3-dihydroxy-4-chlorobiphenyl, and 2-hydroxy-3-methoxy-4-chlorobiphenyl. These metabolites account for approximately 60, 7, 23 and 3 percent, respectively, of the radioactivity extracted from the excreta of treated rats.

3. The major metabolite (90%) of 4,4'-dichlorobiphenyl has been isolated and identified as 3-hydroxy-4,4'-dichlorobiphenyl. Traces of the 2-hydroxy-4,4'-dichlorobiphenyl have also been isolated.

4. The metabolites of 2,4,5,2',5'-pentachlorobiphenyl have been described and are 3-hydroxy-2,4,5,2',5'-pentachlorobiphenyl; 3,4-dihydro-3,4-dihydroxy- and 3,4-dihydroxy-pentachlorobiphenyls. Only approximately 13% of this compound is excreted as the parent compound, 60% of the radioactivity excreted was in the form of the monohydroxy and about 25% was in the form of the dihydrodiol and dihydroxy metabolites.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The polychlorinated biphenyls are ubiquitous environmental contaminants, and knowledge of their biotransformation and the identification of their metabolites is essential to our understanding of their importance as potential hazards to man and his environment. We have evidence that epoxides are formed in the metabolism of PCBs. Epoxides have been implicated in a number of undesirable biological reactions including teratogenesis, carcinogenesis, and tissue necrosis.

PROPOSED COURSE: We are currently looking at the metabolism of 3,5,3',5'-tetrachlorobiphenyl. This PCB has the minimum chlorination which does not allow two adjacent unsubstituted carbon atoms. We feel that two adjacent unsubstituted carbon atoms are a prerequisite to a facile formation of arene oxides which we think are intermediates in the formation of the metabolites we have isolated and identified. In the near future we hope to study the

metabolism of 2,4,5,2',4',5'- and 2,3,6,2',3',6'-hexachlorobiphenyls as the final step in our efforts to understand the effect of degree and position of chlorination on PCB metabolism.

PUBLICATION

Chen, P. R., McKinney, J. D., and Matthews, H. B.: Metabolism of 2,4,5,2',5'-pentachlorobiphenyl in the rat: Qualitative and quantative aspects. Drug Metab. Disp., in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80015-03 PB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Transport of Xenobiotics by Blood and Its Constituents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Matthews	Research Chemist	PB	NIEHS
	M. W. Anderson	Senior Staff Fellow	EBB	NIEHS

COOPERATING UNITS (if any)

Environmental Biometry Branch

LAB/BRANCH

Pharmacology Branch

SECTION

Pharmacokinetics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The polychlorinated biphenyls (PCBs) and certain chlorinated pesticides are readily taken up by the blood and its components and transported to the liver where they are readily released. Our studies suggest that these compounds are distributed between the red blood cells, albumin, and lipoproteins at ratios which vary according to the lipophilicity of the chlorinated hydrocarbon involved. Thus, the process is thought to be a passive one in which the physical and chemical properties of the chlorinated hydrocarbons determine the blood constituent in which they will be most concentrated.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The blood is responsible for the transport of many endogenous substances throughout the body. It also carries either free or bound to its constituents most of the xenobiotics to which the organism is exposed. These studies were designed to first determine the mechanism of xenobiotic transport and secondly to determine if this transport in any way interferes with the normal transport of endogenous materials.

METHODS EMPLOYED: The distribution of ^{14}C-labeled xenobiotics such as biphenyl, four different polychlorinated biphenyls (PCBs), three chlorinated pesticides, one nonchlorinated pesticide, and an endogenous compound, a steroid hormone are being studied in various blood fractions. Methods employed included adsorption and molecular sieve chromatography, density gradient centrifugation, and electrophoresis.

MAJOR FINDINGS: 1) Initially a large portion (up to 60%) of an iv dose of the PCBs was associated with the red blood cells. 2) The remaining 40% of an iv dose of the chlorinated compounds was distributed between the albumin and lipoproteins. 3) Red blood cells released their associated PCBs most rapidly followed by the lipoproteins. 4) The percent of the total dose of a xenobiotic found to be associated with the blood lipoproteins is a function of its chlorination (or lipid solubility).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Environmental exposure of man to a large number of xenobiotics via air, water, or food contamination is significant. These materials are transported by the blood to various detoxication, excretion, or storage sites within the body. Thus, if the transport of any one of these materials should interfere with the normal transport of endogenous materials by the blood then it could result in an imbalance of homeostasis and serve as a mechanism of toxic effect.

PROPOSED COURSE: Current plans call for similar studies of PCB metabolites and several more non-chlorinated compounds and studies of the effect of chlorinated and non-chlorinated exogenous compounds on the blood transport of endogenous compounds, e.g., certain hormones.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80016- 03 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pharmacokinetics of Chlorinated Xenobiotics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. B. Matthews	Research Chemist	PB	NIEHS
	M. W. Anderson	Senior Staff Fellow	EBB	NIEHS
OTHER:	N. Morales	Visiting Fellow	PB	NIEHS
	R. L. Dedrick	Section Chief	BEIB	DRS
	R. Lutz	Chemical Engineer	BEIB	DRS

COOPERATING UNITS (if any)

Chemical Engineering Section, BEIB, DRS, NIH

LAB/BRANCH
Pharmacology Branch and Environmental Biometry Branch

SECTION
Pharmacokinetics

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	0.9	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Polychlorinated biphenyls (PCBs) are rapidly removed from the blood and initially stored in the liver and muscle. At later time points the less chlorinated PCBs are metabolized and excreted while the highly chlorinated PCBs are translocated from the liver and muscle to the skin and adipose tissue which are the sites of long-term storage. The degree and rate of metabolism and the ultimate biologic half-life of PCBs is determined by the degree and position of chlorination. The degree of PCB metabolism decreases as the chlorine content increases, and the effect of increasing chlorine content is most marked when the chlorine atoms are arranged so that the biphenyl molecule does not have two adjacent unsubstituted carbon atoms.

PROJECT DESCRIPTION

OBJECTIVES: To develop a pharmacokinetic model which will predict for a number of species the distribution, accumulation and excretion of polychlorinated biphenyls (PCBs) and other chlorinated xenobiotics after different dose levels and routes of exposure.

METHODS EMPLOYED: The distribution, accumulation, and excretion of four ^{14}C-labeled PCBs (4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl) were studied after iv and oral administration to rats. The major tissues and excreta were sampled at times varying from a few minutes to several weeks posttreatment. Distribution and excretion were studied under conditions of normal feeding and starvation. Analyses were facilitated by the use of a biological materials oxidizer and liquid scintillation counting. All of the data were subjected to further analysis by computer.

MAJOR FINDINGS: 1. Each of the PCBs was rapidly removed from blood with initial storage primarily in the liver and muscle. Long-term storage was in the skin and adipose tissue with most of the stored material being unchanged compounds. Storage of metabolites in skin and adipose tissue accounted for a negligible portion of the total PCB dose. Where two adjacent carbon atoms were present, the excretion of the PCBs was inversely proportional to their degree of chlorination.

2. We will soon complete studies of the distribution and excretion of 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl in the mouse.

3. We have completed our studies of the distribution and excretion of an acute dose of 3,5,3',5'-tetrachlorobiphenyl in the rat. The half-life of this compound in the rat is slightly greater than that of the pentachlorobiphenyl which we studied previously, thus lending support to our theory that two adjacent unsubstituted carbon atoms facilitate PCB metabolism.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Chlorinated xenobiotics are among the most persistent of our environmental contaminants. These compounds are known to cause a variety of biological disorders in man as well as being at least partially responsible for the declining numbers of certain species of wildlife. Accumulation of high concentrations in the tissues of animals exposed to relatively low doses is a characteristic of certain chlorinated xenobiotics. Yet many of these compounds are used in our current methods of food production, disease control and certain industrial processes. Thus, we need to be able to predict what will be the disposition of these compounds in animals and man. Understanding the pharmacokinetics of PCBs in different species will help in this prediction or extrapolation of animal data to man.

PROPOSED COURSE: 1. Disposition studies will be done after treating animals with a chronic low dose until plateau levels of PCB and metabolite tissue concentration are reached. One group of animals will be sacrificed at the plateau and a second group will be held in order to study the decay from plateau levels. The amount of covalent binding (to protein and DNA) will be measured at various time points during the chronic feeding study. The amount of covalent binding will be a measure of the formation of activated metabolites.

2. Disposition studies for two more hexachlorobiphenyls and one more tetrachlorobiphenyl will be done in the rat and mouse. From the disposition information obtained for these eight PCBs, structure-disposition correlations will be attempted for the PCBs in rat and mouse.

PUBLICATIONS

Matthews, H. B. and Anderson, M. W.: The distribution and excretion of 2,4,5,2',5'-pentachlorobiphenyl in the male rat. Drug Metab. Disp. 3: 211-219, 1975.

Matthews, H. B. and Anderson, M. W.: Effect of chlorination on the distribution and excretion of polychlorinated biphenyls. Drug Metab. Disp. 3: 371-380, 1975.

Matthews, H. B., Domanski, J. J. and Guthrie, F. E.: Hair and its associated lipids as an excretory source for chlorinated hydrocarbons. Xenobiotica. In press, 1976.

Matthews, H. B. and Anderson, M. W.: PCB chlorination versus PCB distribution and excretion. Proceedings of the National Conference on PCBs, Chicago, November 1975. In press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80021-02 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Membrane Glycoproteins in Normal and Neoplastic Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	T. Abe	Visiting Fellow	PB	NIEHS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

Glycoproteins in membranes have an important role as receptors for the regulation of growth and metabolism. The enzyme nicotinamide adenine dinucleotide glycohydrolase (E.C.3.2.2.5) was studied in order to utilize a known isolatable membrane glycoprotein as an eventual means for measuring the specific modifications that occur with such proteins during neoplasia. This enzyme was solubilized and isolated from both microsomes and plasma membranes of rat liver homogenates. The compositional analysis of the microsomal enzyme revealed the presence of approximately 12 percent sugar, most of which consisted of neutral hexoses. Multiple activity peaks were observed for the microsomal enzyme by isoelectric focusing. This profile was markedly different from that observed for the plasma membrane enzyme. However, when both of these proteins were treated with an enzyme that removes sialic acid from glycoproteins, a single coincident peak was observed by isoelectric focusing. We conclude from these experiments that the sugar composition of this glycoprotein enzyme is determined by subcellular localization, or vice versa, and that no compositional differences are likely to be observed in the polypeptide backbone of the glycoprotein isolated from either membrane preparation.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: Glycoproteins of cell surfaces apparently have an important role in antigenicity, cell growth, cytodifferentiation, and cellular recognition. The objectives of the present study are to elucidate the biochemical, immunochemical, and structural properties of intact membrane glycoproteins, especially those of the plasma membrane, from both normal and neoplastic cells. Presently, we are studying the enzyme nicotinamide adenine dinucleotide glycohydrolase (E.C.3.2.2.5) which has a high specific activity in the plasma membrane fraction obtained from rat liver homogenates.

METHODS EMPLOYED: Amino acid analysis, polyacrylamide gel electrophoresis, ultracentrifugation, sucrose linear gradient density centrifugation, isoelectric focusing.

MAJOR FINDINGS: NAD glycohydrolase was solubilized from a preparation containing rat liver microsomal and plasma membranes as judged by suitable enzyme markers. Solubilization was achieved by a combination of sonication, freeze-thawing, and digestion with crude pancreatic lipase. Although the lipase preparation was essential for solubilization, it appears that the lipase per se is not the solubilizing enzyme in the crude pancreatic preparation. Approximately 80% of the membrane-bound enzyme was solubilized. The enzyme in the supernatant of the digest was then purified by DEAE-cellulose and Sephadex G-150 columns and then by isoelectric focusing using a pH 6-8 ampholine. Multiple peaks were obtained on focusing using a pH of 7.8. Using this peak, we obtained a 1000-fold purification of the enzyme. This protein was found to have a m.w. of about 83,000 daltons, as measured using sucrose gradient centrifugation. The pI 7.8 enzyme protein was stained with periodic acid-Schiff reagent on electrophoresis gels. The protein contained about 10% hexoses (of the total dry weight), 0.5% hexosamines, and 1-1.5% sialic acid.

Polyacrylamide gel electrophoresis of the pI 7.8 enzyme was essentially homogeneous and could be stained faintly with periodic-acid Schiff reagent, confirming the previous detection of carbohydrates. No unusual amino acid composition was observed.

Plasma membranes were isolated from the rat livers and studied for NAD glycohydrolase activity. In comparison with microsomes the plasma membranes had a 5- to 6-fold greater specific activity for this enzyme. The solubilized enzyme from plasma membranes had a m.w. similar to that of microsomes but on isoelectric focusing the major peak was observed at pI 6.8.

Treatment of solubilized plasma membrane or microsomal NAD glycohydrolase with neuraminidase at pH 5.6 for six hours yielded a single enzyme with a pI of about 9.4. These data suggest that the multiple peaks observed on isoelectric focusing could be due to different amounts of sialic acid residues. Those peaks observed at the lower pI's should have higher amounts of sialic acid. As expected, the pI 6.8 peak from plasma membranes migrated faster on polyacrylamide gels than the pI 7.8 peak isolated from microsomes.

However, we have not been able to purify to electrophoretic homogeneity the enzyme from plasma membranes.

These present findings support a "vectorial" synthesis of glycoproteins. That is, prior to incorporation into the plasma membrane, microsomal NAD glycohydrolase is further transglycosylated with sialic acid. The incorporation of this terminal sugar onto the sugar chain of the enzyme might confer properties that allow for selective cellular localization or conformation with a particular membrane.

Histologic examination of a Fischer 2-344 transplantable hepatoma revealed, unfortunately, a heterogeneous cell population and thereby precluded studies with this tissue.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: There is much evidence to suggest that aberrations at the surface of cells might be responsible for loss of controlled growth or "contact inhibition." These aberrations could occur as modified, blocked, or deleted functional groups of proteins or intact macromolecules. Changes in the specific sugar sequence or "sugar code" could introduce aberrations in proteins. Membranes, as a result of a point mutation of a single protein, can be affected drastically by a cascade of events because they are highly ordered and condensed organelles. The present studies have been undertaken to give basic knowledge about a class of proteins in membranes of normal and tumor cells; these proteins have been strongly implicated as having regulatory roles in metabolism and growth. We believe that accurate observations and clarification of the aberrations of tumor cells rests with the understanding of the biochemistry and structure of intact membrane molecules.

PROPOSED COURSE: We are presently measuring half cystine content of the microsomal enzyme and also comparing kinetically the microsomal and plasma membrane enzymes. At the completion of these experiments, the project will be submitted for publication and the project terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80022-03 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Hepatic Transglutaminase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. DiAugustine	Research Chemist	PB	NIEHS
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Hepatic transglutaminase has been previously shown to catalyze the coupling of many primary amines to proteins. This enzyme is also able to catalyze the cross-linking of certain peptide chains. In the present study, this enzyme in the cytosol fraction of rabbit liver in the presence of calcium couples amines, such as putrescine, to endogenous proteins. The enzyme also cross-links endogenous proteins in the cytosol fraction to yield proteins with a very high molecular weight. The enzyme was isolated and purified from the cytosol fraction. The purified enzyme was able to cross-link proteins of purified rabbit liver plasma membranes. Very high concentrations of amines were required to inhibit this reaction. It is suggested that a physiological role of this enzyme might be the cross-linking of intracellular proteins and that this reaction might be regulated by calcium.

PROJECT DESCRIPTION

OBJECTIVES: Transglutaminase is an enzyme of unknown function in the liver. This enzyme, however, can couple (covalently) in vitro selected amines such as phenethylamine, isoniazid, and putrescine to certain endogenous proteins. Calcium is required for this reaction. This enzyme activity appears operative in plasma as Factor XIII for the catalysis of the ε(γ-glutamyl)lysine bonds between fibrin monomers. Factor XIII prior to the sequential clotting reactions exists -- unlike the liver enzyme -- as a zymogen in plasma. There is some evidence to indicate that the hepatic enzyme might function in vivo in the manner of Factor XIII by catalyzing ε(γ-glutamyl)lysine bonds for membrane proteins (P.J. Birkbichler et al. Biochim. Biophys. Acta 291: 149, 1973).

The objectives of the present program are to 1) purify and to characterize rabbit liver transglutaminase, 2) determine what proteins in the cytosol and membrane fractions of the hepatocyte can act as acceptors for amines such as putrescine, spermine, isoniazid, and tyramine; 3) determine any role of Ca^{++} or other divalent metals in the stimulation of hepatic intracellular transglutaminase. Unlike the blood enzyme (Factor XIII) hepatic transglutaminase needs no proteolysis to initiate activity. However, it is difficult to explain how transglutaminase is operative in vivo when intracellular calcium is so very low in concentration ($\sim 1 \times 10^{-6}$ M), and 4) to identify any intracellular proteins which are cross-linked by hepatic transglutaminase and to examine any structural or dynamic roles of those proteins.

METHODS EMPLOYED: Column chromatography, liquid scintillation counting, isoelectric focusing, polyacrylamide gel electrophoresis, ultracentrifugation, amino acid analysis, cesium chloride gradient centrifugation.

MAJOR FINDINGS: 1. The enzyme was purified by precipitation with acetic acid (pH 5.0), $(NH_4)_2SO_4$ fractionation, and by DEAE-Sephadex and Sephadex G-100 column chromatography. The molecular weight of the enzyme was between 83,000 and 87,000 daltons as estimated by Sephadex G-100, Sepharose 4B gel filtration with 6M guanidine HCl, SDS-gel electrophoresis, and amino acid composition. This protein has a partial specific volume of 0.717, an $E^{1\%}_{cm}$ (280 nm) of 14.0 and a pI of 5.35 as obtained by isoelectric focusing. Amino acid composition of rabbit liver transglutaminase was similar to that of the guinea pig liver enzyme (Folk, J. E. [1966], J.B.C. 241, 5518) except that the rabbit preparation had significantly more half-cystine residues. Optimal conditions for enzyme activity measured by hydroxylamine incorporation into CBZ-glutaminyl glycine were 37°C and pH 6.5. The Km value of this reaction was 33.3×10^{-3} M. The enzyme also had esterase (p-nitrophenyl acetate) activity (Km = 0.2×10^{-3} M) which was previously shown for the guinea pig enzyme. In both reactions, calcium was required for enzyme activation.

2. Cell cytosol obtained from homogenates of rabbit livers which were perfused extensively with isotonic sucrose will readily couple labeled amines, such as putrescine, glycine ethyl ester, isoniazid or tyramine, to an endogenous (cytosol) acceptor. This reaction is calcium-dependent and can be blocked with iodoacetamide. A sulfhydryl-reducing agent was not found to be required for maximum incorporation in the experiments using cytosol as an enzyme source and as an amine acceptor. Incorporation could be further enhanced by adding to cytosol a partially purified transglutaminase preparation which contained no acceptor protein for enzymatic coupling of amines. Following extensive dialysis and subsequent cesium chloride equilibrium, density ultracentrifugation; all the labeled material was associated with the protein band.

Cytosol acceptor proteins as determined by calcium-stimulated amine labeling were also separated by gel-filtration on Sephadex G-150 columns after dialysis. The major acceptor protein fraction was associated with the void volume. No free amines were detected in the Ve peak and no amines could be released after prolonged dialysis against 5M KCl. Isoelectric focusing (pH 3.5-10) revealed multiple isotopic (^{14}C-putrescine-labeled) peaks with virtually all the activity between pH 3.5-5. As with the gel filtration experiments, the isoelectric focusing experiments showed no direct correlation between the amount of amine binding and the amount of protein per fraction.

The cytosol of E. coli or S. epidermidis, which was found to have no transglutaminase as determined by the NH_2OH-CBZ-glutaminylglycine reaction, failed to demonstrate the calcium-dependent coupling of any amines to an endogenous acceptor.

3. Cytosol incubated in the presence of calcium (5 mM) yielded a densely staining band that would barely migrate into 6% SDS-polyacrylamide gels. The formation of this band could be blocked by including iodoacetamide (5 mM) in the reaction mixture. Formation of this band was partially inhibited by putrescine or glycine ethylester. The addition of any of these chemicals following incubation failed to inhibit formation of this band. Densiometric tracings of the Coomassie-stained gels revealed that the cytosol incubated with calcium gave diminished or weaker staining of several peptide subunits of very large molecular weight (> 200,000). These subunits appeared to be less than one percent of the total cytosol protein.

When cytosol obtained from perfused rabbit lung was incubated with calcium, much less cross-linking was observed, i.e., there was much less stained protein at the top of the SDS-gels when compared to that formed by the liver cytosol reaction. Further studies showed that also unlike the liver, lung cytosol had relatively very low amine-coupling to endogenous protein and very low activity using the NH_2OH-CBZ-glutaminylgylcine reaction.

4. Plasma membranes isolated from rabbit liver failed to demonstrate the formation of "extensively-cross-linked" protein when incubated in the presence of calcium. However, we were able to demonstrate this cross-linking only when the membranes were incubated with purified rabbit liver transglutaminase, calcium, and cysteine. Iodoacetamide effectively blocked the

formation of this newly cross-linked protein observed at the top of SDS gels. As with liver cytosol, the bands disappearing during incubation of the complete reaction mixture to apparently form the newly cross-linked band at the top of the gel were those of very high molecular weight, at least greater than 200,000 daltons.

5. In light of these results, we are postulating that 1) the amine coupling to endogenous protein acceptors and the formation of extensively cross-linked proteins observed with liver cytosol are reactions catalyzed by the same enzyme, transglutaminase. 2) That the inhibition of the cross-linking reaction by amines suggests that some of the active centers of proteins of high molecular weight which were to participate in this reaction were, instead, labeled covalently with the amine. In accord with this, the transglutaminase-catalyzed coupling of amines to fibrin has been shown to inhibit formation of ε(γ-glutamyl)lysine cross-links of this protein. (Lorand et al. BBRC, 25, 629, 1966). 3) The large size of the peptides that are observed on SDS-polyacrylamide gels both in membranes and cytosol and the apparent further cross-linking of these peptides by transglutaminase strongly suggests that the ε(γ-glutamyl)lysine bond are responsible for the formation of these very large subunit structures. Unlike the disulfide cross-links, the ε(γ-glutamyl)lysine bond would be stable to reduction by SDS-mercaptoethanol.

Although Birckbichler et al. (BBA, 291, 149, 1973) did not examine any particular group of cellular proteins on the basis of size, they, nevertheless, gave chemical evidence for the presence of the dipeptide ε(γ-glutamyl) lysine bond in eucaryotic cells. The fact that further cross-linking is possible in vitro for these excessively large peptides is compatible with the fact that transglutaminase-catalyzed cross-linking of fibrin monomers seldom proceeds to absolute completion (Doolittle et al., BBRC, 44, 94, 1971). On SDS-polyacrylamide gels this phenomenon can be observed as a time-dependent progression of subunits of about 80,000 daltons to material so extensively cross-linked that it does not enter the gel. (Schwartz et al. J. Biol. Chem., 248, 1395, 1973).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

1. The fact that a number of amine-containing drugs such as isoniazid and hydralazine can be coupled to certain proteins by transglutaminase makes this enzyme a suitable candidate for drug- or chemical-induced antigen formation in vivo. Some clinical evidence has already been reported suggesting that antibodies to plasma transglutaminase are responsible for isoniazid-induced hemorrhaging.

2. Transglutaminase offers a limited method for coupling selected compounds to proteins for use in radioimmune assays.

3. Neoplastic cells apparently produce higher levels of polyamines, which can be detected in the serum. Evidence also suggests that the polyamines, especially putrescine, are coupled to cell membrane proteins which can be detected immunochemically. We suggest transglutaminase as a catalyst for such a reaction.

4. The ε(γ-glutamyl)lysine cross-link is a recently discovered covalent protein-protein interaction that appears to be ubiquitous in mammalian cells. If this bond can be definitely ascertained in hepatocyte plasma membranes, it would give us a new and important component to consider in the study of the physical, chemical, and functional properties of membranes. What process controls the catalysis of the cross-link in membrane? Calcium influx to the cell? From where are the proteins derived to make the cross-link? What property does this cross-link give to membranes?

PROPOSED COURSE: 1) To further determine the biochemical and physical properties of rabbit liver transglutaminase, 2) to identify soluble and membrane proteins capable of acting as acceptors for transglutaminase-catalyzed reactions. On completion of these experiments, the results will be submitted for publication and the project terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80023-04 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Composition and Origins of the Acellular Lining Layer of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Senior Staff Fellow	PB	NIEHS
	R. P. DiAugustine	Research Chemist	PB	NIEHS
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COOPERATING UNITS (if any)

Environmental Biology and Chemistry Branch

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The terminal airways of the lung are lined with an acellular layer of material which appears vital in the maintenance of normal pulmonary function. The composition and origins of the acellular lining layer are being investigated. Current attention has been directed towards: (1) the secretion of pulmonary surfactant, and (2) the acellular lining layer as an actively metabolizing medium.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: Numerous chemical analyses of the acellular lung lining layer have been made and there is little doubt concerning the identity of the surfactant material obligatory for normal lung function. However, the acellular layer is also a barrier to the environment and consequently may be one of the first sites subject to attack by inhaled toxic agents. Unfortunately, apart from its lipid composition very little is known about the acellular lining layer.

The objectives of this investigation are as follows: (1) to determine the enzymatic and protein composition of the lung lining, (2) to determine the origin of these activities, and (3) to investigate compositional variations of the lining under the influence of drugs, disease, and airborne pollutants.

METHODS EMPLOYED: Acellular lining layer material is obtained by lavaging the lungs via the trachea. Particulate materials are isolated from the lavage by isopycnic centrifugation on sucrose gradients. Lamellar bodies as found in alveolar Type II cells are isolated using differential centrifugation. Enzyme and protein analyses are carried out using polyacrylamide gel electrophoresis and isoelectric focusing.

MAJOR FINDINGS: The secretion of lamellar bodies by Type II cells is clearly indicated from studies involving the incorporation of ^{32}P-labeled inorganic phosphate into phospholipids of the lamellar bodies and acellular lining layer. The inability of isolated lamellar bodies to catabolize phospholipids shows that the structures are not heterophagosomes in which material phagocytized from the acellular lining layer by the Type II cell is undergoing degradation. Other studies of isolated lamellar bodies indicate the presence of selected lysosomal hydrolases (e.g., β-N-acetyl-glucosaminidase and α-mannosidase) and that these enzymes are secreted into the acellular lining layer by the Type II cell.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The composition of the acellular lining layer of the lung is complex consisting of enzymes, proteins, and lipids. The origins of these many components are obscure. Our studies provide evidence that the Type II cell is involved in the secretion of not only phospholipids but hydrolytic enzymes as well.

The acellular lining layer of the terminal airways is vital for normal lung function. The influence of inhaled environmental toxicants on this layer is not known, but in many environmentally related pulmonary diseases (e.g., silicoalveolar proteinosis), the composition of the acellular lining layer appears to be altered. The involvement of the acellular layer in the progression or mediation of pulmonary disease is not known. Information relating to the composition and origins of the acellular lining layer and its variation following inhalation of toxicants is of immediate concern to occupational and environmental health.

PROPOSED COURSE: The properties of the protein components of the acellular lining are being investigated. The investigation of the selective secretion of enzymes to the lung lining are being extended to the human as pulmonary lavage samples become available. Effects of pollutant exposures on the acellular lining layer will be studied in animals.

PUBLICATIONS

Saru, S., DiAugustine, R. P., and Lynn, W. S.: Lipids in the lung lavage of patients with alveolar proteinosis and in rabbit lung lamellar organelles. Ann. Rev. Resp. Dis. In press (1976).

Hook, G. E. R. and DiAugustine, R. P.: Secretory cells and the extracellular lining layer of the pulmonary terminal airways. Env. Health Perspectives. In press (1976).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80025-02 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Interaction of Aromatic Polycyclic Hydrocarbons with Macromolecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. E. Voytek	Senior Staff Fellow	PB	NIEHS
OTHER:	R. P. DiAugustine	Research Chemist	PB	NIEHS

COOPERATING UNITS (if any)

Laboratory of Chemistry, NIAMDD, NIH

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project was to study the interaction of polycyclic aromatic hydrocarbons (PAH) (specifically benzo[a]pyrene and 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene) with RNA and protein from rat liver and lung microsomes. PAH were incubated with microsomal preparations, and PAH and metabolites bound to RNA and protein were solubilized and isolated by solvent extraction followed by thin layer and column chromatography and gel electrophoresis. Similar metabolites of PAH are formed by both liver and lung, although liver appears to be metabolically more active than lung for metabolism of the PAH studied.

PROJECT DESCRIPTION

OBJECTIVES: To study the interactions of polycyclic aromatic hydrocarbons (specifically benzo[a]pyrene and 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene) with RNA and protein from microsomes. Attempts will be made to separate and isolate hydrocarbon-bound RNA and protein species. Separation and characterization of ^{14}C-labeled metabolites will also be attempted.

METHODS EMPLOYED: 1) Isolation of microsomes from rat liver and lung. 2) Incubation of ^{14}C-labeled hydrocarbons with microsomal preparations. 3) Solubilization, separation, and isolation of hydrocarbon-bound RNA and protein species via extraction with ethyl acetate and phenol followed by thin-layer chromatography, column chromatography, and gel electrophoresis.

MAJOR FINDINGS: Preliminary studies have involved developing and testing appropriate procedures for separating and isolating carcinogen-bound RNA and protein species. The first procedure involved an ethyl acetate extraction of the microsomes followed by thin-layer chromatography of the ethyl acetate phase. The aqueous layer was extracted with phenol. Subsequently, the resulting two phases were analyzed by gel electrophoresis. Thin-layer chromatography has shown that both benzo[a]pyrene and 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene are metabolized to similar products by both the lung and liver tissues. Estimations of the activity of the tissues indicate that the liver is metabolically more active, although it has been reported in the literature that, on the basis of the protein content of the tissues, rat lung is as active as rat liver. Gel electrophoresis has not yielded anything conclusive.

The second procedure that has been developed involves a phenol extraction of a microsomal resuspension followed by 1) application of the aqueous phase to a Sephadex column to separate out the RNA's, and 2) application of the phenol phase to a Bio-Gel column to separate out the proteins. Activity peaks from the columns are then further characterized by gel electrophoresis. This second procedure has just been initiated.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Benzo[a]pyrene, an polycyclic aromatic hydrocarbon, is a widespread environmental carcinogen which produces tumors in lung tissue. Like many chemical carcinogens, benzo[a]pyrene is metabolized by microsomal enzymes to electrophilic alkylating agents which can react with cellular components. Several potentially reactive metabolites are formed as a consequence of microsomal metabolism but recent evidence suggests that it is the 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene-8,10-oxide product which is mainly responsible for the mutagenic and oncogenic properties of the parent hydrocarbon.

Because microsomal enzymes metabolize many compounds, such as benzo[a]pyrene to more reactive forms, macromolecules associated with the microsomes could be the first cellular components exposed to the newly formed alkylating agents.

One of the main functions of the microsomes is the synthesis of proteins, a process requiring both RNA and protein components. If interaction occurs between alkylating agents and macromolecules, the function of the macromolecules could be altered such that protein synthesis occurs abnormally or is disrupted altogether. The effects of such changes with respect to cellular transformation and carcinogenesis is unclear.

Since 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene is the more immediate precursor of the carcinogen 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene-9,10-oxide, examination of its metabolism by lung microsomes and the covalent binding properties of its metabolites to microsomal RNA's and proteins may provide some insight about the effects of chemical carcinogens on these organelles. Furthermore, benzo[a]pyrene has never been shown to cause hepatomas in animal experiments. Therefore, it would be of interest to compare the metabolism of 7,8-dihydro-7,8 dihydroxybenzo[a]pyrene and binding of its metabolites to microsomal macromolecules in lung and liver tissue.

PROPOSED COURSE: Termination due to departure of the principal investigator.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80026-03 PB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

DNA Repair Synthesis in Mouse Oocytes and Zygotes -- Radiation and Carcinogen Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. E. Voytek	Senior Staff Fellow	PB	NIEHS
	K. Ku	Visiting Fellow	PB	NIEHS
OTHER:	L. A. Moustafa	Senior Staff Fellow	ETB	NIEHS

COOPERATING UNITS (if any)

Environmental Toxicology Branch

LAB/BRANCH

Pharmacology Branch

SECTION

Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project was to establish whether mature mouse oocytes can repair damage caused by agents known to cause lesions in DNA and to assess the effects of these agents on the developmental process. Our results have shown that mature mouse oocytes are capable of performing DNA repair synthesis. However, in spite of this capability, one-cell embryos are very sensitive to low doses of UV light and ionizing radiation. None of the one-cell embryos developed past the two-cell stage. Both forms of radiation greatly accelerated fragmentation and degeneration of the cells. However, treatment of one-cell embryos with the carcinogen, NA-AAF, only reduced the number of embryos that developed to the blastula stage and did not increase the rate of fragmentation or degeneration. In contrast, exposure of two-cell embryos to low doses of UV light, ionizing radiation, or NA-AAF did not block development to the blastula stage. Our findings suggest that a target for radiation other than DNA exists in one-cell embryos and this target apparently is necessary for first cleavage to occur.

PROJECT DESCRIPTION

OBJECTIVES: 1) To establish direct evidence for DNA repair synthesis in secondary oocytes and fertilized eggs and 2) to assess physical and chemical carcinogen effects on the development of the fertilized ova.

METHODS EMPLOYED: Isolation of secondary oocytes and fertilized eggs after superovulation, autoradiography, and culturing of embryos.

MAJOR FINDINGS: Our results have shown that mature mouse oocytes are capable of performing DNA repair synthesis. However, in spite of this capability, one-cell embryos are very sensitive to low doses of UV light and ionizing radiation. None of the one-cell embryos developed past the two-cell stage. Both forms of radiation greatly accelerated fragmentation and degeneration of the cells. However, treatment of one-cell embryos with the carcinogen, NA-AAF, only reduced the number of embryos that developed to the blastula stage and did not increase the rate of fragmentation or degeneration. In contrast, exposure of two-cell embryos to low doses of UV light, ionizing radiation, or NA-AAF did not block development to the blastula stage. Our findings suggest that a target for radiation other than DNA exists in one-cell embryos and this target apparently is necessary for first cleavage to occur.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Man today is exposed to an increasing number of chemical and physical hazards which can result in genetic damage, which in turn could lead to cellular mutations, transformation, carcinogenesis, decrease in survival time, and death. Therefore, acquiring knowledge of genetic repair mechanisms capable of correcting such damage is extremely important. However, little effort has been expended on the repair processes in germ cells, and, in particular, oocytes and fertilized eggs. The importance of DNA repair during early cell stages of development may be greatly magnified over repair found in somatic cells because of the many time sequences in events that may be gene regulated. It would seem that damaged genetic material would have to be rapidly repaired or the development of the embryo would cease or would proceed abnormally. Therefore, the influence of many potential toxic agents, either physical or chemical, on the repair processes during early developmental stages could present hazardous consequences.

PROPOSED COURSE: Terminated. Principal investigators have left NIEHS.

PUBLICATIONS

Ku, K. Y., Moustafa, L. A., and Voytek, P.: Induced DNA repair synthesis by UV radiation in mature mouse oocytes arrested in metaphase II. Intern. Research Communication Service 3: 607, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80027-02 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Alterations in the Glucagon Receptor-Adenylate Cyclase System in Hepatomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. D. Mirel	Staff Associate	PB	NIEHS
OTHER:	R. P. DiAugustine	Research Chemist	PB	NIEHS

COOPERATING UNITS (if any)
Department of Endocrinology, UNC; NCI; Howard University

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

An investigation was made of the mechanism responsible for the loss of glucagon-stimulated adenylate cyclase in poorly differentiated hepatomas. Adenylate cyclase activity and ^{125}I-glucagon binding were measured in the plasma membrane preparations over a wide range of glucagon concentrations. Neither glucagon-stimulated adenylate cyclase activity nor ^{125}I-glucagon binding could be detected in these plasma membranes. Detergent-solubilized membranes from these hepatomas also did not bind glucagon, whereas the solubilized liver membranes bound up to 1.2 picomoles of ^{125}I-glucagon. In well-differentiated hepatoma membranes, adenylate cyclase activity and ^{125}I-glucagon binding were present. Fluoride and epinephrine-stimulated adenylate cyclase activity in all hepatomas was similar to that observed in normal liver membranes. We conclude that there is no detectable glucagon receptor in these undifferentiated tumors.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: Glucagon is a key hormone in the regulation of glucose homeostasis. Probably its most important site of action is the liver where it stimulates glycogenolysis and gluconeogenesis. Glucagon action appears to be initiated by attachment of glucagon to a specific cell surface receptor which is in some way coupled to membrane-bound adenylate cyclase. Adenylate cyclase is the key enzyme involved in cyclic-AMP production. In several hepatomas induced by carcinogens, there is a loss of adenylate cyclase response to glucagon. One of the main objectives of this project is to elucidate the mechanism of loss of the glucagon-induced adenylate cyclase response in various hepatomas. Is there actual loss of the glucagon receptor or is there masking of the receptor? Or possibly is the defect not in the receptor but in coupling (or transduction) of the receptor to adenylate cyclase.

METHODS EMPLOYED: Tumor transplantation, liquid scintillation, column chromatography, gamma counting, sucrose density gradients, ^{125}I-glucagon binding.

MAJOR FINDINGS: We have looked at four transplantable hepatomas originally induced by various chemical carcinogens. This study deals with the mechanism responsible for the loss of glucagon-stimulated adenylate cyclase activity in poorly differentiated hepatomas. Plasma membranes were prepared from homogenates of two well-differentiated hepatomas (Morris rat 7787 and Dalton mouse 9815), two poorly differentiated hepatomas (Morris rat 7288-C and Dalton mouse 129), and normal liver. Adenylate cyclase activity and ^{125}I-glucagon binding were measured in the plasma membrane preparations over a wide range of glucagon concentrations. Neither glucagon-stimulated adenylate cyclase activity nor ^{125}I-glucagon binding could be detected in the poorly differentiated hepatomas. Fluoride and epinephrine-stimulated adenylate cyclase activity in all hepatomas was similar to that observed in normal liver. In the well-differentiated hepatomas, adenylate cyclase activity and ^{125}I-glucagon binding were present, although half-maximal stimulation for both cyclase activity and binding required higher levels of glucagon than did normal liver. In addition, maximum activity of adenylate cyclase and maximum binding of glucagon were less than that of liver. Plasma membranes from liver and hepatomas were solubilized with Lubrol-PX. After reducing the concentration of detergent, the membranes were incubated with ^{125}I-glucagon and then chromatographed on a column of Bio-Gel, 1.5m. Two peaks containing both protein and ^{125}I-glucagon were found for liver but not for the poorly differentiated hepatomas. Fractions from the Bio-Gel column containing the greatest concentrations of protein were also subjected to a microbinding assay. Material from the poorly differentiated tumors did not bind glucagon in this system, whereas the solubilized liver membranes bound up to 1.2 picomoles of ^{125}I-glucagon per mg of protein. We conclude that there is no detectable glucagon receptor in these undifferentiated tumors.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Investigations of alterations of cell surface receptors in neoplastic tissues might lead to the understanding of the molecular events which are responsible for the loss of growth control of cells. Recently there have been several reports of carcinogen-treated animals in which there have been alterations in hormone-mediated adenylate cyclase before there were any histologic changes. It is presumed that these changes reflect alterations in hormone-receptor interaction. Although the initiation of chemical carcinogenesis may depend on the interaction of the carcinogen (or its metabolites) with cellular nucleic acids, the actual loss of growth control, cellular contact inhibition, or metastasis is thought to be due to some epigenetic function or sequence of reactions initiated at the surface of cells. The biochemical or structural events responsible for the loss of the glucagon receptor might bear analogous correlates to those events or macromolecules which are lost or blocked at the cell surface and are important in growth regulation.

PROPOSED COURSE: The major portion of the work regarding the glucagon receptor has been completed and submitted for publication. Currently, we are investigating the possibility that proteolytic activity of the hepatomas may play a role in the destruction of the receptor itself--as a secondary phenomenom. We intend to look at the reversibility of the receptor destruction with protease inhibitors. We are also interested in exploring the possible role of calcium ion in the change in receptor function.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80028-01 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Diagnostic Markers of Pulmonary Damage and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Senior Staff Fellow	PB	NIEHS
	M. J. Reasor	NIH Postdoctoral Fellow	PB	NIEHS
	D. Nadeau	Visiting Fellow	PB	NIEHS
OTHER:	S. E. Fabro	Guest Worker	ETB	NIEHS

COOPERATING UNITS (if any)
Inhalation Toxicology Section, Environmental Biology and Chemistry Branch; Department of Medicine, Duke University; Columbia Hospital for Women, Department of Gynecology and Obstetrics, George Washington University

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.2	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the identification and characterization of components which are lung specific or pulmonary disease-related which could be used as diagnostic markers for pulmonary damage or disease. Current attention is being focused on: 1) the soluble alkaline phosphatases of the normal lung, 2) abnormal hydrolases present in pulmonary lavage effluents from patients with alveolar proteinosis, and 3) enzymic components of human amniotic fluid which originate in the fetal lung.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The objectives are as follows: 1) to identify lung-specific enzymes and proteins which are released into serum or the pulmonary airways following the production of cellular damage by inhaled toxicants, 2) to investigate and characterize unusual enzymes or proteins present in diseased lungs, and 3) to identify enzymes or proteins in human amniotic fluid which originate from the fetal lung and to investigate the use of such components to predict fetal lung maturity.

METHODS EMPLOYED: Enzyme investigations involve the use of gel-filtration chromatography, polyacrylamide gel electrophoresis, isoelectric focusing on polyacrylamide gels, immunoelectrophoresis, and differential centrifugation. Amniotic fluid is obtained during Caesarian delivery of term mothers. Pulmonary lavage effluents from patients with alveolar proteinosis is obtained as a by-product of the therapy essential to the well-being of these patients. The lavage procedure involves instillation of isotonic saline into the left or right lung of the patient followed by drainage.

MAJOR FINDINGS: 1. Markers of Pulmonary Damage. The release of organ-specific soluble alkaline phosphatases (AP) into the blood from diseased or damaged organs has prompted considerable interest in these isoenzymes as diagnostic tools. APs from a number of organs have been partially characterized, but AP of the lung has received little attention. The rabbit lung (L) was found to contain two soluble AP isoenzymes which, on linear gradient polyacrylamide gels (4-30% acrylamide), have apparent molecular weights of about 185,000 and 220,000 Daltons. As much as 12% of the total soluble AP of the lung is recovered from the extracellular lining layer (ELL) of the lung by pulmonary lavage. Only a 185,000 molecular weight enzyme is present in the ELL. The APs of the soluble fraction ($214{,}000 \times g_{av}$, 60 min. supernatant) of lung homogenates and ELL were compared with those of the small intestine, liver, bone, kidney, spleen, and serum. The electrophoretic profiles of AP from these tissues were different from L-AP and ELL-AP, although a 185,000 molecular weight AP was present in bone and serum. In addition, isoelectric focusing on polyacrylamide gels indicated that the L-AP and ELL-AP were inactivated by heating at 55°C at rates different from each other and different from the other tissue APs. These results indicate that the complement of alkaline phophatases present in rabbit lung and in the extracellular lining layer is different from that present in the other tissues studied and may be specific for the lung.

2. Disease-Related Pulmonary Components. Alveolar proteinosis is a disease of unknown etiology characterized by the accumulation of large amounts of proteinaceous material in the acini of the lungs. We have found a soluble alkaline phosphatase (AP) with unusually high apparent molecular weight in pulmonary lavage effluents of patients with alveolar proteinosis. Following sedimentation of particulate material ($214{,}000 \times g_{av}$, 60 min.) from lavage effluents, a soluble phase was isolated which contained AP activity (13.2 ± 5.9 nmoles p-nitrophenylphosphate hydrolyzed/min/mg

protein [$\bar{X}$ ± SD, n = 4]) and protein (7.6 ± 1.2 g protein/6 liters of effluent). The AP has been purified 245-fold using gel filtration and the enzyme appears to be present only as a high molecular weight complex (M.W. > 20 x 10^6 Daltons) as judged by its exclusion from Sepharose Cl-4B. Treatment of the complex with n-butanol or Triton X-100 results in the release of a relatively low molecular weight AP (M.W. ~ 200,000) which migrates as a single enzyme when electrophoresed on 4% polyacrylamide gels and linear gradient (4-30%) polyacrylamide gels. AP, as found in the high molecular weight complex, has a pH optimum of 9.9 at 37°C and is stimulated maximally (77%) by the presence of 1.25 mM $MgCl_2$. The presence of this unusual alkaline phosphatase in large quantities in the airways of patients with alveolar proteinosis suggests that the enzyme may be potentially useful as a diagnostic marker and monitor of the disease.

3. Pulmonary Components in Amniotic Fluid. Fetal lung derived components have been sought in amniotic fluid obtained during Caesarian delivery of full-term infants. Phospholipid-rich lamellar body-like structures have been isolated from the amniotic fluid. Morphologically, these structures resemble lamellar bodies of the alveolar Type II cell and lamellar body derived structures often seen in the extra-cellular lining layer of adults' lungs. In normal humans lamellar bodies are found associated only with the lung, and consequently there is little doubt that the lamellar body-like structures found in amniotic fluid have arisen from the fetal lung.

A number of hydrolytic enzymes previously found associated with lamellar body related structures isolated from the pulmonary extracellular lining layer of rabbits have been detected in the structures isolated from human amniotic fluid. The nature of these enzymes and structures is at present under investigation.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:
1) Many human pulmonary diseases have been described but unfortunately the diagnosis of these diseases is not usually made until the disease is well advanced. X-ray methods generally are not capable of detecting pulmonary diseases excepting in the advanced stages and even then are often incapable of distinguishing between diseases. Methods for the diagnosis of pulmonary disease and the detection of pulmonary damage in the earliest possible stages are needed most urgently.

The rabbit lung has been shown to contain two lung-specific soluble alkaline phosphatases. The presence of either of these enzymes in serum following exposure of the animal to a toxicant would be indicative of pulmonary damage.

Based upon preliminary analyses made on lavage effluents from patients with other pulmonary diseases as well as in three healthy human volunteers, the unusual soluble alkaline phosphatase present in lavage effluents from patients with alveolar proteinosis may be unique to this disease. Consequently, the reliance on morphological studies of lung biopsy samples for the diagnosis of alveolar proteinosis may yet be replaced by simple segmental lavage using fiberoptic bronchoscopy with little danger to the patient.

2. Lamellar body related structures derived from fetal lung and directly involved in a vital pulmonary function have been isolated from amniotic fluid. Enzymes associated with these lamellar structures should allow a simple means of estimating fetal lung maturity prior to birth.

Respiratory distress syndrome or hyaline membrane disease, which is the major cause of morbidity and mortality among premature infants, is associated with inadequate production of pulmonary surfactant. Lamellar body-related structures as found in the pulmonary airways appear to be a form of pulmonary surfactant. At present reliable tests for the estimation of pulmonary surfactant present in amniotic fluid are cumbersome since they involve chromatographic procedures. An enzyme assay for fetal lung maturity would be of major significance to clinicians as well as provide a means by which the effects of environmental pollutants on lung maturation could be studied.

PROPOSED COURSE: These studies clearly indicate the lung-specific nature of the soluble alkaline phosphatases. The use of these enzymes as markers of pulmonary damage will be investigated following the exposure of rabbits to toxicant gases known to produce lung damage (such as 100% O_2, NO_2, O_3, and SO_2). Both serum and lavage effluents will be investigated for the presence of alkaline phosphatase isoenzymes.

The unusual alkaline phosphatase present in large quantities in lavage effluents from patients with alveolar proteinosis shows considerable promise as a marker for this disease. Detailed characterization and further purification of the complex is planned and underway.

Lamellar-body associated enzymes present in amniotic fluid will be compared with those found free in the fluid. Correlations between lamellar bodies, selected enzyme components, fetal lung maturity, and gestational age will be attempted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 ES 80029-01 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Investigations of Human Pulmonary Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. R. Hook	Senior Staff Fellow	PB	NIEHS
OTHER:	M. J. Reasor	NIH Postdoctoral Fellow	PB	NIEHS
	D. Nadeau	Visiting Fellow	PB	NIEHS

COOPERATING UNITS (if any)

Department of Medicine, Duke University

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

These studies have been concerned with a number of human pulmonary diseases of possible environmental origin, which are treated clinically by broncho-alveolar lavage. The diseases being studied are alveolar proteinosis, bronchial asthma, and some occupationally related unnamed pulmonary disorders. At present, attention has been directed towards characterization of lavage effluent components from patients with alveolar proteinosis, a human pulmonary disease of unknown etiology characterized by the accumulation of large amounts of proteinaceous material in the acini of the lungs.

PROJECT DESCRIPTION

OBJECTIVES: The objectives are as follows: 1) to characterize particulate and soluble materials present in lavage effluents according to their morphology, enzymic, protein, and lipid content; 2) using animal models to determine the cellular and subcellular origins of these materials; and 3) elucidate possible mechanisms leading to the establishment of the disease.

METHODS EMPLOYED: Broncho-alveolar lavage effluents from patients with pulmonary diseases are obtained from the Department of Medicine at Duke University. The isolation of particulate components from the lavage effluents involve differential and isopycnic centrifugation procedures.

MAJOR FINDINGS: The quantities of particulate material present in the airways of patients with alveolar proteinosis vary with the patient but commonly range from 20-100 g per lung (15-60 g protein, 5-40 g phospholipid). Particulate material present in lavage effluents is morphologically complex. It does not resemble particulate material present at the lung lining of healthy animals such as rabbits and consequently does not appear to represent hypersecretion of a normal extracellular lining layer component.

Attempts have been made to isolate components from the lavage effluents, and four morphologically complex but distinct fractions have been obtained using velocity sedimentation centrifugation. The association of enzymes with these subfractions represents what may prove to be of major significance in determining the origins of the particulate material and clarifying the nature of the disease.

Isopycnic centrifugation of a 10,000 x g sediment on continuous sucrose density gradients indicates the particulate material consists of four major components with densities of approximately 1.08, 1.18, 1.19, and 1.22. β-N-acetylglucosaminidase and α-mannosidase are found associated with the lowest density material (d = 1.08) indicating the presence of lamellar body material. These two enzymes have been found associated with lamellar body structures from the airways of normal rabbits. The high density components (d > 1.17) are characterized by the presence of both acid and alkaline phosphatases and 5'-nucleotidase indicating similarities with endoplasmic reticulum or plasma membrane.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The study of most pulmonary diseases is confined to animal models which vary enormously in their approximation to the human condition. The study of human pulmonary diseases at the biochemical level is severely restricted by the limited and limiting availability of tissues. The availability of fresh materials as by-products of patient therapy offers a unique opportunity for the study of a few human pulmonary diseases of possible environmental origin.

A number of human pulmonary diseases are characterized by the accumulation of material in the airways of the lung. The nature of these materials and their origins are obscure. Characterization of materials which are manifestations of a disease state may provide valuable information about the processes which give rise to that particular disease condition.

PROPOSED COURSE: The characterization of materials present in pulmonary lavage effluents from patients with alveolar proteinosis will continue. Eventually, animal models of this disease will be investigated which would allow the investigation of biochemical processes leading to the disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 ES 80030-01 PB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
The Formation of Polypeptide Hormones by Chemically-Induced Lung Carcinomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. DiAugustine	Research Chemist	PB	NIEHS
OTHER:	R. D. Mirel	Staff Associate	PB	NIEHS

COOPERATING UNITS (if any)
Medical Center, Duke University; Dept. of Pathology, University of North Carolina

LAB/BRANCH
Pharmacology Branch

SECTION
Molecular Pharmacology and Biochemistry

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Certain non-endocrine tumors have been reported to produce polypeptide hormones. For instance, the formation of adrenocorticotropic hormone (ACTH) and "big" or precursor ACTH by lung tumors in man is well known. The proposed studies will attempt (1) to correlate the synthesis of ACTH as detected by radioimmunoassay and immunohistochemical techniques with the various stages of tumor growth, (2) to examine the possible formation de novo of polypeptide hormones in vitro in tracheal organ cultures in the presence of chemical carcinogens, and (3) to develop sensitive radioimmunoassay procedures that can be used for the diagnosis, management, and early detection of bronchogenic carcinomas.

PHS-6040
(12-75)

PROJECT DESCRIPTION

OBJECTIVES: The formation of substances by non-metastatic tumors which are not normally detectable in the adult parent cell or organ is now very well documented in the literature. For instance, carcinoembryonic antigen and alpha-foetoprotein are found in the serum of some patients or animals bearing neoplasms. The resection of these tumors usually leads to marked reductions of these substances in serum, suggesting that the tumor is capable of expressing genes heretofore repressed. Many tumor biologists or oncologists consider this "ectopic" formation of proteins to be an almost obligate but variable manifestation observed with all invasive carcinomas.

The recognition of the association of Cushing's syndrome with carcinoma in lung has led to the identification of ACTH, for instance, in extracts of tumors resected from these patients. Recently, it has been shown that blood levels of ACTH were elevated above normal in patients with carcinoma of the lung without clinical evidence of Cushing's syndrome. Studies have shown that the major form of ACTH secreted by various lung carcinomas was the "big" or precursor-form of ACTH. This "big" ACTH is cross-immunoreactive with small ACTH (the normal pituitary form), but it has negligible bioactivity, which might account for the absence of Cushing's syndrome in many patients in spite of elevated hormone levels. Bronchogenic tumors have also been demonstrated to synthesize vasopressin, parathyroid hormone, and human chorionic gonadotropin. As with the "ectopic ACTH syndrome," the hypersecretion of these polypeptide hormones were suspected when overt symptoms of endocrine malfunction were present clinically. Pronounced symptoms of ectopic secretion are considered to represent late stages of tumor growth.

With the development of very sensitive methods, such as radioimmunoassay, to detect specific polypeptides or proteins, it should now be possible to measure low enough levels of these hormones prior to the onset of invasive carcinoma or the full-blown symptoms associated with endocrine hypersecretion.

The further refinement for measuring or understanding the patterns for polypeptide hormone secretion by lung tumors is the subject of this project. In light of the insensitive indicators such as X-rays or scans, the proclivity of bronchogenic carcinoma to produce polypeptide hormones could offer a suitable assessment of the stage of tumor growth and, moreover, the detection of the premalignant lesion.

METHODS EMPLOYED: Tracheal organ cultures, radioimmunoassay, gamma counting, polyacrylamide gel electrophoresis, column chromatography, liquid scintillation, fiberoptic bronchoscopy.

MAJOR FINDINGS: This project has just begun in collaboration with clinical researchers at Duke University.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: It has been acknowledged that perhaps 90 percent of human cancers have an environmental component(s) in their etiology. Cancers of pulmonary origin are associated with various environmental causative agents (air pollution, asbestos, smoking, etc.) and are among the most resistant of all tumors to therapeutic measures. Conventional therapy (e.g., surgery and radiation) might be more successful if such tumors could be detected at an earlier stage of development. Development of markers for earlier detection of pulmonary tumors is consistent with the aspect of our mission relating to the development of better test systems for detecting and evaluating early toxic effects of environmental chemicals. The major goal of the above-described endeavors will be to develop more sensitive and more meaningful methods to accurately assess pulmonary tumors. To achieve this end it is vital that we clarify or predict the associative production of ectopic peptides with specific cytological changes.

PROPOSED COURSE: This proposed study of the ectopic production of polypeptides by lung tumors is essentially designed to operate at two related levels. One level will encompass a basic laboratory effort to study the possible formation de novo of polypeptide hormones using cultures of tracheal rings or epithelium. At another level, we will apply known or improved methods for the detection of ectopic proteins or peptides in the lung lavage of patients who are suspected to have or are developing a lung carcinoma.

At first, we will use transverse sections of rabbit trachea. These rings can be maintained for weeks or months as a well-differentiated epithelium in culture using many different media. Suitable chemical carcinogens such as diethylnitrosamine or benzpyrene will be used to produce cellular transformation. A recent report showed that hyperplasia with high rates of DNA synthesis were observed in cultures having a continued exposure to benzpyrene at 1 µg/ml. Two reports have shown that squamous metaplasia and hypersecretion can develop in cultures lacking appropriate concentrations of vitamin A. Thus, it appears that the tracheal epithelium in culture can undergo some, if not all, of the typical histological changes observed in vivo.

Assuming, then, that we are able to routinely prepare and maintain these pre-neoplastic or neoplastic lesions in culture, we shall attempt to detect, using radioimmunochemical methods, the presence of polypeptide hormones in the media or in extracts of cultured organ homogenates. Initially, we shall measure radioimmunochemically any presence of ACTH in vitro. However, we also intend to use similar methods to check for the presence of HCG and PTH polypeptide hormones. We shall also investigate the production of labeled unknown peptides by culturing normal or neoplastic trachea with isotopic leucine and separating any labeled peptides by gel filtration. Sephadex G-50 columns, for instance, have been shown to give marked separation of "big" ACTH (M.W. ~ 20,000) and "small" ACTH (M.W. ~ 4,500).

We shall be especially interested in correlating the amount or type of peptide with the observed cellular changes occurring with the tracheal pseudostratified columnar epithelium. These structural studies will be done in collaboration with Dr. Johnston and his colleagues in the cytopathology laboratory at Duke Medical Center.

The radioimmunoassay procedures in progress or other suitable detection methods developed to measure ectopic peptides in the respiratory tract should allow us to routinely examine sputum, material collected from lung lavage or fiberoptic bronchoscopy or biopsy speciments. This material will be obtained with the cooperation of Drs. J. Kylstra, P. Pratt, and A. Spock, Duke Medical Center. We shall be especially interested in examining lung secretions of high risk groups, such as smokers or asbestos workers, for the presence of ectopic peptides.

In addition, collaborative studies on specimens of lavage fluid obtained from lungs of patients with lung carcinoma will be undertaken with Dr. Alexander Spock, Department of Pediatrics, Duke Medical Center. Dr. Spock has been studying the production, release, and metabolism of various mediators, including histamine, prostaglandins, slow reactive substances, serotonin and bradykinin. An investigation of the role of these bioactive substances would be extended to patients with carcinoma of the lung. If the lung is the site of various reactions ending with the liberation of bioactive agents, the result should be an undesirable appearance of these mediators in the systemic circulation as well as within the tracheobronchial tree. Hopefully, the current study of chemical mediators in lavage fluid will provide some understanding of the interrelationship between chemical mediators and the lung. Furthermore, Dr. Spock will study the ectopic production of polypeptides by lung tumors.

CONTRACTS

BATTELLE PACIFIC NORTHWEST LABORATORIES - RICHLAND, WASHINGTON (NIH-72-2043)

TITLE: "Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants"

CONTRACTOR'S PROJECT DIRECTOR: R. E. Wildung, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D.

DATE CONTRACT INITIATED: January 1, 1972

CURRENT ANNUAL LEVEL: $98,000

PROJECT DESCRIPTION

OBJECTIVES: The proposed investigations will aim at determining, by means of radiotracers and advanced chemical analytical techniques, the fate of agriculturally important metals in soils, in soil microorganisms, and in representative grass and broadleafed plants grown on treated soil. The rate and extent of uptake and translocation and the sites of deposition of metallic compounds within the plant will be measured. In microbial and plant tissues in which radioactivity is accumulated, investigations will be directed toward determining the bond types and the chemical forms of the metabolites. Establishment of the deposition locations of metals and metal transformation products will allow both the reaction pathway to be estimated and the rate-limiting reactions to be studied in depth. The results of the fundamental investigations will be utilized to devise recommendations for chemical treatments and soil-crop management practices which may serve as remedial or preventive measures to minimize health hazards arising from the accumulation of metals or their metabolites.

METHODS EMPLOYED: Ritzville soil was thoroughly mixed with labeled and unlabeled isotopes. A split-root (soil/nutrient solution) method was employed to grow soybeans to maturity in the metal containing soils. The plants were cultured in a growth chamber under constant light (14 hr light; 10 hr dark), temperature (27½C light; 20½C dark) and humidity (40-45%). At maturity the plant tops and roots were harvested (133 and 142 days from planting). Plants were separated into cotyledons, roots, stems, leaves, immature bean pods, mature bean pods and mature beans. In all cases the tissues were analyzed for total metal radioisotopes.

With regard to the microbial studies, major emphasis was placed on the characterization of those organisms that exhibited high resistance to metals using various enrichment techniques at different metal concentrations. In the initial studies viable counts were used as a measure of growth, in the more recent work turbidimetric and dry weight measurements have been used.

MAJOR FINDINGS: Initially, methods were developed and applied to the measurement of the optimum uptake and sites of deposition of 17 metals in shoots

from sequential harvests of barley (Hordeum vulgare) and soybeans (Glycine max) grown in soils containing radiolabelled metals at several concentration levels. In addition, chemical techniques were developed and applied to determination of the solubility, molecular weight distribution, charge and thin-layer chromatographic characteristics of 17 metals in plant tissues. These 17 metals were: silver (Ag), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), tin (Sn), thallium (Tl), tungsten (W), and zinc (Zn). Where radioactivity associated with the metals was below detection levels, metal-ligand exchange techniques were employed to identify metal binding ability of isolated plant components. An ancilliary part of these studies involved measurement of the effects of metals in soil on the synthesis of plant components containing functional amino groups.

Of this group, Cu, Fe, Mn, Mo, and Zn are required in small amounts for normal plant growth. Only Cu and Mo caused plant growth reductions when added to soil. Cd, Co, Ni, and W were taken up in significant quantities but did not cause growth reduction. In particular, Cd and Ni were accumulated in significant amounts in the bean. Tl, As, Ag, Hg, and Pb were toxic to the plants. Thallium in particular, was taken up in large quantities which accumulated in the plant stem. In addition, the Tl was bound to material with less than 500 MW.

Concurrently with the plant studies, investigations were undertaken to determine the biochemical fate of the 17 metals in soils used for plant culture. Initially, methods were developed and applied to the determination of the effects of metals at different concentration levels on changes with time in the numbers of aerobic and anaerobic bacteria, aerobic and anaerobic sporeforming bacteria, actinomycetes and fungi, and on changes in soil respiratory activity. During the course of the microbial studies, changes in the solubility of the metals in soil were determined as a function of incubation time. Detailed investigations of the mechanisms of metal inhibition on microbial processes were also undertaken with selected metals.

From the results of the above-described investigations, 9 metals were selected for intensive study as those exhibiting the most potential for biochemical alteration in the soil and uptake and concentration by plants. These metals were As, Cd, Cr, Hg, Mo, Ni, Ag, Tl, and W.

The split-root technique for plant culture, in combination with autoradiographic and radiochemical analytical techniques used in previous studies, was employed to determine the distribution of the selected metals in the roots, stems, leaves and fruiting bodies of the soybean (Glycine max). Plant components containing the highest metal concentrations were subjected to chemical analyses to determine the form of the metal in tissues. Separation procedures previously developed for low molecular weight (<10,000 and <500) components were employed and extended to include detailed characterization and identification of separated metal metabolites. Lower molecular weight substances which were more likely to enter the food chain received initial emphasis; however, techniques were developed for fractionation of higher molecular weight (>10,000) components. Final characterization and identification of

components of either the high or low molecular weight fractions were accomplished using a broad range of analytical techniques including thin-layer chromatography, gas chromatography - mass spectrometry, infrared or ultraviolet spectroscopy, and electron-spin resonance.

Using the enrichment techniques previously developed, investigations were undertaken to isolate, identify, and study in vitro microorganisms exhibiting resistance to metals in the soil employed in plant studies. It is these organisms which have the greatest potential for biochemical modification of metals in soil. Bacteria, fungi, and actinomycetes isolated from soil enrichments which use metals as selective agents will then be evaluated as to their ability to form organometallic complexes from inorganic metal salts.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A number of avenues exist by which metals may enter the human food chain. The principal direct avenue probably results from using metals in agriculture as pesticides, desiccants or as contaminants of fertilizers. The importance of soil and plants in this pathway has been underscored by the spectre of metal conversion to more soluble and more toxic heavy metal complexes which may subsequently be taken up by plants and thereby enter directly into human foodstuffs. Particular attention should be given to determining the mechanisms for converting inorganic metals to organic complexes and the extent of this conversion. This information should provide a sound basis for estimating the potential hazard arising from the continued use of these materials and may provide the key to remedial measures.

PROPOSED COURSE: On the basis of knowledge accumulated to date, it is proposed that research emphasis be placed on the metals, thallium, chromium, nickel, and cadmium, which are important pollutants associated with energy and food production. Efforts will be directed principally toward (i) biochemical characterization of the form of cellular and exocellular organometal complexes produced by microorganisms isolated from soil on the basis of their resistance to metals, (ii) characterization of the soluble organometallic complexes in soil using chemical techniques developed in microbial studies, (iii) determination of the plant availability of microbial metabolites in nutrient culture, (iv) measurement of the kinetics of plant uptake of metals from soil, and the extent of mobilization or remobilization to the seed during growth of soybeans to maturity, (v) biochemical characterization of the form of the metal in the plant using techniques similar to those employed in the characterization of metal form in microbial and soil studies, with particular emphasis on those classes of compounds shown to be available to the plant in nutrient culture and metals which exhibit a propensity to localize in the seed.

In addition, a test system is being developed which will allow the investigators to vary a number of important soil parameters and study the influence of these variables on the uptake of the metal by the plant.

KEYWORD DESCRIPTORS: heavy metals, soybeans; uptake; distribution in plant components; soil microbial interaction.

BROOKHAVEN NATIONAL LABORATORY - UPTON, NEW YORK
(Interagency Agreement)

TITLE: "Chemical and Environmental Mutagen Studies Utilizing the Tradescantia Test System"

CONTRACTOR'S PROJECT DIRECTOR: A. H. Sparrow, Ph.D.

PROJECT OFFICER (NIEHS): F. J. de Serres, Ph.D.

DATE CONTRACT INITIATED: June 6, 1972

CURRENT ANNUAL LEVEL: $168,000

PROJECT DESCRIPTION

OBJECTIVES: The objectives of the proposed research are: (a) to demonstrate the usefulness of and further develop the Tradescantia somatic mutation test system as a quantitative monitor in environments containing known or suspected mutagens; (b) to use special genetic clones of Tradescantia to establish the relative mutagenicity of a limited number of mutagens (primarily in gaseous form), as to magnitude the effect, specificity of action and possibility of reversion; (c) to look for evidence of genetic repair of DNA injury produced by chemical mutagens and to look for possible threshold levels below which induced mutation rate is negligible and (d) to determine in greater detail the time and dose response for several loci for one selected gaseous mutagen, comparing these with presently available radiation data and with results observed in animal cells.

METHODS EMPLOYED: Two different clones of Tradescantia (clone 02 and clone 4430), which are heterozygous for a flower petal color mutation were used to determine the mutagenicity of exposure under various experimental conditions. Refinements in chemical mutagen exposure techniques were made to establish 1,3-dibromoethanol (DBE) as the mutagen standard in these studies rather than ethylmethane sulfonate (EMS) and this work has continued using tritium labeled DBE. Plant breeding and new clone selection has also been continued to obtain more sensitive clones which might provide better indicators of weak mutagens and chronic levels of exposure. New clones with a wider range of spontaneous mutation rates at each of three loci are being used along with existing mutable clones to develop methods for studying repair.

MAJOR FINDINGS: Additional exposures of longer duration have been made with DBE; dose response curves from exposures of concentrations of 2 to 100 ppm for 2 to 144 hours exposure show that straight line relationships on a log-log plot with slopes of about 2. Furthermore, the concept of time X concentration plotted against mutation frequency also shows a straight line relationship on a log-log plot for all exposures from 2 hours to 144 hours.

^{3}H-DBE has been utilized to determine the tissue dose of DBE. Because of the beta emission, a given dose of mutagen labeled with ^{3}H-DBE induced a mutation frequency that is about 5 times that induced by unlabeled DBE. Radioautography has shown that ^{3}H-DBE is not localized in the cell. The use of labeled DBE is

also being planned to determine whether penetration of DBE following exposure will be slower in clone 02 than the sensitive clone 4430.

Exposures to new gaseous mutagenic chemicals were made with CO, SO_2 and vapona. CO was tested for 30 hours at a level of 50 ppm and was also weakly mutagenic. Exposures to vapona (2,2-dichlorovinyl dimethyl phosphate), however, showed no mutagenic effect.

Major emphasis during this period was directed towards the development of a mobile laboratory for field studies.

Work has also continued to evaluate new strains to select those with better flower color as well as those with increased sensitivity to mutation induction.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract should provide a badly needed assay for mutagenic airborne environmental chemicals. It is extremely sensitive to ionizing radiation, and the exploratory work with selected chemical mutagens during this past year suggests that it may be able to detect concentrations as low as 10 ppm. This assay system should provide a meaningful assay for the production of gene mutations and relates to the Institute's program to develop sensitive assay systems to detect the mutagenic activity of environmental chemicals.

PROPOSED COURSE: At the conclusion of the present course of oxidant exposures, work will focus on determining the relative mutagenicity of specified industrial compounds using DBE as the standard. Plans are also being made to deploy the mobile laboratory to make it possible to assay airborne environmental pollutants at field stations in the New York City area where NERC-EPA is measuring ozone, SO_2 and NO_2 levels.

COLLEGE PARK METALLURGY CENTER, U. S. BUREAU OF MINES (IAA-14-09-0070-598)

TITLE: "Mineralogical Characterization of Fibers"

CONTRACTOR'S PROJECT DIRECTOR: Dr. William Campbell

PROJECT OFFICER (NIEHS): Dr. John A. Moore

DATE CONTRACT INITIATED: August 20, 1975

CURRENT ANNUAL LEVEL: $300,000

PROJECT DESCRIPTION

OBJECTIVES: To advise and assist NIEHS in the development of protocols to be used in the preparation and characterization of mineral fibers. To assume a lead role in the chemical, physical, and mineralogical characterization of these materials.

To prepare and provide in 1200 pound quantities a crocidolite and two sample amosite materials.

METHODS EMPLOYED: Standard mineralogic methodology.

MAJOR FINDINGS: The contractor is characterizing the tremolite sample provided; it has negotiated an agreement with Ontario Research Foundation, Canada, for the preparation of the amosite and crocidolite samples.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The NIEHS is responsible for assessing the biological effects of ingested asbestos. The ultimate application of animal studies is extrapolation of results to human exposure. Exposure of the U. S. population to asbestiform material varies as to asbestos type as well as the fiber size. It is vital for interpretation of the public health consequence of ingested asbestos to have rigidly characterized materials with which to conduct animal studies.

PROPOSED COURSE: These studies will continue.

KEYWORD DESCRIPTORS: Asbestos; crocidolite; amosite.

Cornell University
Division of Biological Sciences
Ithaca, New York 14850
NO1-ES-5-2100

TITLE: "Effect of Chronic Administration of Toxaphene on Hepatic Enzyme Induction and Circulating Steroid Levels

CONTRACTOR'S PROJECT DIRECTOR: David Peakall, Ph.D.

PROJECT OFFICER (NIEHS): R. M. Philpot, Ph.D.

DATE CONTRACT INITIATED: September 1, 1974

CURRENT ANNUAL LEVEL: Terminated

PROJECT DESCRIPTION

OBJECTIVES: Determine the effect of chronic toxaphene administration to rats on the hepatic drug-metabolizing system and circulating steroid levels.

METHODS EMPLOYED: Plasma estrogen, progesterone, and testosterone levels will be assayed by radioimmunological techniques. Drug-metabolizing enzymes will be assayed by standard procedures using the 18,000 x g supernatant fraction from liver.

MAJOR FINDINGS: Toxaphene was found to increase liver weight, this increase becoming significant by the fifth day. Hepatic enzyme induction was marked, a significant increase again occurring by the fifth day. There is a distinct tendency for enzyme activity to fall with prolonged exposure and values obtained at three and six months are significantly lower than those obtained at 15 days. Testosterone levels were lower at day 5 ($p = 0.02$) but had returned to control values by day 15 and did not significantly change thereafter. No alterations of the levels of lactic or pyruvic acid were found in the chronic experiments. The tissue levels of toxaphene were similar at both three and six months. For liver the mean residue level was 26 ppm (wet weight) and in the brain 11 ppm.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This research relates to the efforts of NIEHS to determine the magnitude and significance of the hazard to man's health inherent in long-term exposures to low level concentrations of chemical environmental agents. This research also represents an integral portion of the US-USSR Environmental Health Program.

PROPOSED COURSE: Project terminated.

THE DOW CHEMICAL COMPANY, MIDLAND, MICHIGAN (NIH-NIEHS-73-2102)

TITLE: "Effect of Environmental Components on Reproduction and Ontogenesis."

CONTRACTOR'S PROJECT DIRECTOR: B. A. Schwetz, D.V.M., Ph.D.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: February 5, 1973

CURRENT ANNUAL LEVEL: $357,000

PROJECT DESCRIPTION

OBJECTIVES: (1) To determine the teratogenic potential in animals of environmental components when administered singly or in combination; (2) to determine species differences in response to teratogens and to study the etiology of such differences; and (3) to investigate the mechanism of action of teratogens and to improve experimental testing and extrapolation to man.

METHODS EMPLOYED: In general, the screening approach outlined by the World Health Organization (Technical Report No. 364, 1967) is being followed with certain modifications to alleviate some of the problems encountered by private industry during the testing of potential therapeutic agents.

MAJOR FINDINGS: The teratogenic response previously demonstrated following water deprivation during pregnancy occurred in mice that were given water ad libitum but pair-fed to water deprived mice. These results indicated that the teratogenicity seen probably stemmed from the reduced food intake associated with water deprivation rather than from the water deprivation per se. SKF-525A and piperonyl butoxide were not effective inhibitors of microsomal enzymes in rabbits as indicated by hexabarbital sleeping time after single or multiple administration by the i.p. or oral routes of the MTD during the period of major organogenesis. In mice, both inhibitors were effective at dose levels not causing overt maternal toxicity or teratogenicity.

Neither carbon monoxide (up to 250 ppm/day) nor alcohol (25% in drinking water) were teratogenic in rabbits or mice.

Carbaryl administration to mice by gavage and by diet at the MTD did not reveal overt teratogenicity even though embryotoxicity was present. In rabbits, 200 mg Carbaryl/kg/day from Days 6 through 18 of gestation resulted in both maternal and fetal toxicity and in an increased incidence of umbilical hernias among the offspring.

Combinations of dichlorvos with SKF-525A, piperonyl butoxide, phenobarbital, alcohol, or carbon monoxide did not reveal teratogenic potentiation; hence, even through use of these agents in two species, dichlorvos could not be demonstrated to be a teratogen.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Most environmental agents have not been tested for teratogenic potential. These

studies were undertaken to provide such information on a variety of agents to which man is exposed. Since it is known from studies in laboratory animals that combined administration of two teratogens can potentiate teratogenic response, combinations of non-teratogenic agents were tested to determine whether conditions of increased sensitivity to the developing embryo might be revealed. This information will serve in the formulation of a rational basis for evaluation of the potential hazard to human development produced by the use and exposure of these chemicals.

PROPOSED COURSE: To complete studies currently being conducted, to publish the results, and to test additional agents identified as being of high priority for NIEHS study.

KEYWORD DESCRIPTORS: teratogenesis; SKF-525A; piperonyl butoxide; carbon monoxide; carbaryl; alcohol; phenobarbital; synergism.

GEORGIA INSTITUTE OF TECHNOLOGY - ATLANTA, GA 30332
(NIH-NO1-ES-5-2135)

TITLE: "Development of a Polygenic Assay for Point Mutations in Mice"

CONTRACTOR'S PROJECT DIRECTOR: John W. Crenshaw, Jr., Ph.D.

PROJECT OFFICER (NIEHS): E. R. Soares, Ph.D., Senior Staff Fellow, Mammalian Genetics & Cytogenetics Section, EMB

DATE CONTRACT INITIATED: June 26, 1975

CURRENT ANNUAL LEVEL: $76,500

PROJECT DESCRIPTION

OBJECTIVES: The scope of this contract is to develop an understanding of the mutagenic effects in mice of some known mutagens, as measured by mean and variance changes in polygenic characters.

METHODS EMPLOYED: The contractor proposes to investigate the cumulative effects of multiple mutagen-induced polygenic mutations on a series of traits on F_1 and F_2 generation progenies descended from mutagenized males of an inbred strain of mice. F_1 and F_2 progeny will be examined for a series of traits including: age of eruption of incisors, mean and variance of body weight at weaning, sex ratio at weaning, serum cholesterol level at weaning, and hematocrit at weaning. The contractor proposes to study at least two different mutagens in two strains of mice.

MAJOR FINDINGS: This contract was initiated in June 1975. The first semi-annual report indicated that data collection was proceeding as scheduled and statistical analysis of the collected data will begin shortly.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: In the human environment, there are many compounds known to be mutagenic. At present, no reliable assay exists for determining the rate of induction of point mutations in humans, or more generally, in mammals. Development of a polygenic test system could provide a means for detecting damage of this kind. Furthermore, once developed and understood, such a system would be economically feasible and could be coordinated with other mammalian mutagenesis test systems.

PROPOSED COURSE: The estimated length of this contract is 4 years.

HAZLETON RESEARCH LABORATORIES, VIENNA, VIRGINIA (NO1-ES-5-2158)

TITLE: "Long-Term Study of the Biological Effects of Ingested Asbestos in Rats"

CONTRACTOR'S PROJECT DIRECTOR: Dr. Henry Rutter

PROJECT OFFICER (NIEHS): Dr. John A. Moore

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $749,865

PROJECT DESCRIPTION

OBJECTIVES: The contractor is to perform the following; (1) Determine if life-time ingestion of asbestos is carcinogenic or produces other pathologic effect; (2) Determine the response to asbestos gavage during the neonatal period and the subsequent life-time ingestion of an asbestiform containing diet; (3) Assess the interaction, if any, between a chemical carcinogen known to produce bowel cancer and ingestion of asbestos; (4) Assess these objectives using six specific asbestos or tremolite samples.

METHODS EMPLOYED: A standard life-time bioassay using F-344 rats. Experimental groups will be exposed to asbestos in the diet. The duration of diet exposure will be from neonatal period through the lifetime of the animal. Upon death of the animal, a complete necropsy is to be performed with subsequent histopathologic evaluation of all major tissues.

MAJOR FINDINGS: The contractor is assembling needed caging and other equipment for the study which is to be done under strict microbiological control. The actual animal feeding studies are to commence late in the fiscal year.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The design of this study should allow one to determine: the nature of the biological effects, if any, to ingestion of asbestos; the relationship of fiber length to bioligical effect; if the biological effects are modulated by neonatal exposure or exposure to a chemical carcinogen.

PROPOSED COURSE: These studies will continue.

KEYWORD DESCRIPTORS: Bioassay; asbestos.

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE, CHICAGO, ILLINOIS

(NO1-ES-5-2157)

TITLE: "Long-Term Study of the Biological Effects of Ingested Asbestos in Hamsters"

CONTRACTOR'S PROJECT DIRECTOR: Dr. Mary Henry

PROJECT OFFICER (NIEHS): Dr. John A. Moore

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $230,033

PROJECT DESCRIPTION

OBJECTIVES: Determine if life-time ingestion of asbestos is carcinogenic or produces other pathologic effect in hamsters; assess what interaction may exist, if any, between a chemical carcinogen known to induce bowel cancer and ingestion of asbestos; assess the above objectives using three specific asbestiform types; titrate the carcinogenic and other toxic response to dimethylhydrazine or methylazoxymethanol in order to select that dose of one of these two chemicals in which to subsequently gauge the interaction to this chemical and ingestion of asbestos; to characterize the six asbestos or other mineral materials to be used in the NIEHS ingestion studies using light and electron microscopic technology.

METHODS EMPLOYED: A standard life-time bioassay using hamsters. Experimental groups will be exposed to asbestos in the diet. The duration of diet exposure will be from neonatal period through the lifetime of the animal. Upon death of the animal, a complete necropsy is to be performed with subsequent histopathologic evaluation of all major tissues.

MAJOR FINDINGS: The contractor has been actively evaluating possible asbestiform materials as to fiber size distribution. Dose ranging studies with dimethylhydrazine and methylazoxymethanol were started in late 1975. Studies were also completed which defined the methods that insure complete mixing of asbestos in animal diets and that subsequent pelleting of the diet did not affect fiber characteristics. The actual feeding studies with these materials is expected to commence late in FY-76.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The design of this study should allow one to determine the nature of the biological effects, if any, to oral ingestion of asbestos. To determine if effects are only produced in exposure to a specific type or fiber length of asbestos; in addition, to determine if neonatal exposure or exposure to a chemical carcinogen known to produce bowel cancer is modulated through concomitant or subsequent exposure to ingested asbestos.

PROPOSED COURSE: These studies will continue.

KEYWORD DESCRIPTORS: Asbestos; bioassay; dimethylhydrazine; methylazoxymethanol.

THE JACKSON LABORATORY - BAR HARBOR, MAINE (NIH-NO1-ES-4-2159)

TITLE: "Development of a New Mouse Strain to Maximize the Sensitivity of a Point Mutation Assay"

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D., James Womack, Ph.D.

PROJECT OFFICER (NIEHS): Lawrence R. Valcovic, Ph.D.

DATE CONTRACT INITIATED: February 19, 1974

CURRENT ANNUAL LEVEL: $39,000

PROJECT DESCRIPTION

OBJECTIVES: The sensitivity of a point mutational assay system is directly related to the number of loci that can be tested in each individual. In the present system in our laboratory, nine loci are sampled. Other inbred strains of mice possess enzyme variants which can possibly be incorporated into one of our present strains. This contract seeks to fully identify these additional variants (at least 13) and then by repeated backcrossing to incorporate them into the C57BL/6J genome.

METHODS EMPLOYED: Starch gel electrophoresis is used to determine whether a strain carries an allele that is not found in either the DBA/2J or C57BL/6J strains. When such a variant is found, the strain is backcrossed repeatedly to C57BL/6J for 8-10 generations to develop a new strain which will be homozygous with C57BL/6J. Electrophoresis tests will be performed on progeny in each generation to insure that the allelic differences already present in the C57BL/6J line are maintained.

MAJOR FINDINGS: Several strains carrying new variant alleles have been crossed to the C57BL/6J strain. Most lines have proceeded through the seventh backcross generation with electrophoretic verification of the incorporation of the variant alleles. The following linkage relationships have been established: Np-1 and Es-10 on chromosome 14, Amy-1 and Amy-2 on chromosome 12, and Acp-1 on chromosome 17.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: While many compounds in the environment have been shown to cause point mutations in lower organisms, fewer compounds have been tested in mammals due to the lack of a feasible test system. Also, a program to monitor the human population for the detection of electrophoretic mobility mutations is in the developmental stages. However, no comparable study has been conducted with any laboratory mammal and hence, there is no base-line data for known mutagens for this type of mutational event. In an attempt to elucidate these areas, we are presently using two mouse strains that have nine different isozymes which can be detected electrophoretically. Since maximal sensitivity depends on polymorphic loci, it is desirable to increase the number of enzyme differences between the two strains. There are at least 13 other allelic differences which are present in various other strains or stocks of mice. If these were incorporated into

one of our strains, this would greatly increase the sensitivity of the system with a minimal increase in efforts. The strain will be made available for mutagenesis testing of environmental pollutants, carcinogens and drugs to the scientists in the Environmental Mutagenesis Branch.

PROPOSED COURSE: The offspring obtained from crosses will be analyzed for particular loci and those carrying the desired alleles will be backcrossed to C57BL/6J. The procedure will be carried for 8-10 generations.

THE JACKSON LABORATORY - BAR HARBOR, MAINE 04609
(NIH-NO1-ES-4-2156)

TITLE: "Development of a System for Detecting Lethal Mutations in Mice"

CONTRACTOR'S PROJECT DIRECTOR: Thomas H. Roderick, Ph.D.

PROJECT OFFICER (NIEHS): W. Sheridan, Ph.D., Section Head, Mammalian Genetics and Cytogenetics Section, EMB

DATE CONTRACT INITIATED: January 15, 1974

CURRENT ANNUAL LEVEL: $43,000

PROJECT DESCRIPTION

OBJECTIVES: At present no efficient test system exists for detection of point mutations in mammals. The aim of the contract is to develop strains of mice, carrying marked inversions, which may be used in studies of induction of point mutations. Theoretically these tests will be comparable to the system presently used in Drosophila. The prime goal is to induce inversions in as many chromosomes of the mouse as possible and combine them in a number of strains to be used as tester stocks.

METHODS EMPLOYED: The contractor induces chromosome breaks and inversions by means of radiation and/or chemical agents. He mates the treated animals and in the F_1 does cytological analysis of one testis of each individual male. If a significant increase in anaphase bridges appears, this is taken as an indication that the animal may carry an inversion; and he is mated to get offspring for further study. Following cytological and linkage studies to characterize the aberration, decision is made as to the utility of the inversion and appropriate marker genes are introduced into the stock.

MAJOR FINDINGS: The contractor has induced and recovered 31 inversions. Of these, 14 have been thoroughly characterized for linkage and chromosomal morphology. One of the inversions has been successfully utilized in tests to detect induced recessive lethal mutations.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Recessive lethal mutations are known to exist at high frequencies in human populations. Many environmental compounds are known to be mutagenic and capable of causing both chromosome aberrations and point mutations. With an efficient animal model to study the induction of such mutations, more accurate estimates can be made of the risks such compounds imply to the human population.

PROPOSED COURSE: The estimated length of the contract is five years. Efforts will continue to induce and characterize further inversions. It is proposed to combine inversions with Robertsonian Translocations to increase the effectiveness of the system.

KNOX COLLEGE - GALESBURG, ILLINOIS (NIH-NO1-ES-5-2126)

TITLE: "The Effects of Dietary Deficiency on the Sensitivity to Mutagenic Treatment"

CONTRACTOR'S PROJECT DIRECTOR: Billy W. Geer, Ph.D

PROJECT OFFICER (NIEHS): W. Sheridan, Ph.D., Head, Mammalian Genetics and Cytogenetics Section, EMB

DATE CONTRACT INITIATED: May 1, 1975

CURRENT ANNUAL LEVEL: $44,500

PROJECT DESCRIPTION

OBJECTIVES: In investigations using Drosophila melanogaster cultured on synthetic media deficient in Vitamin E, the frequency of chromosomal effects which were induced by irradiation was increased compared with treated cultures raised on non-deficient media. It is our purpose to investigate whether or not dietary deficiency might change the overall physiological state of the organism making it more susceptible to the actions of mutagenic agents.

METHODS EMPLOYED: Wild-type Drosophila will be raised on a complete, defined, synthetic, germ-free medium. Subcultures will be placed on eleven (11) different media from which specific vitamins and minerals have been singly eliminated or depleted. Flies on deficient media and flies on whole medium will be exposed to similar doses of a known potent chemical mutagen. The frequencies of recessive lethal mutations, dominant lethal mutations and chromosomal aberrations (translocations) will be determined. Comparisons between the treated groups and with concomitant control groups will be made in order to study any differences in the occurrence of induced genetic damage related to the diet.

MAJOR FINDINGS: Sex-linked recessive lethal studies have been completed for two of the deficient diets under study. The mutation frequencies were significantly higher for both the mutagen-treated choline-deficient and nicotinic acid-deficient test males than the frequency observed for mutagen-treated males raised on an optimal diet.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A greater part of the human population on a world-wide basis is known to be subjected to nutritional insufficiency at the present time. At the same time, it is known that there are present in the human environment compounds which are mutagenic. Knowledge of the existence of effects of dietary deficiency on sensitivity to mutation induction will be a necessary consideration in attempts to extrapolate from information derived from laboratory animals in estimating risks to the human population.

PROPOSED COURSE: The estimated length of the contract is two years.

PUBLICATIONS

Geer, B.W. and Reno, D.L.: The Effects of Dietary Deficiencies for Choline and Nicotinic Acid on the Sensitivity of Drosophila melanogaster to Mutagenic Treatment. 7th Annual Meeting of the Environmental Mutagen Society, March 12-15, 1976, Atlanta, Georgia (abstract).

LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
ALBUQUERQUE, NEW MEXICO
(Interagency Agreement Between NIEHS and AEC)

TITLE: "Respiratory Tract Deposition Models"

CONTRACTOR'S PROJECT DIRECTOR: Otto G. Raabe, Ph.D.

PROJECT OFFICER (NIEHS): Phillip J. Walsh, Ph.D.

DATE CONTRACT INITIATED: November 11, 1971

CURRENT ANNUAL LEVEL: $200,000

PROJECT DESCRIPTION

OBJECTIVES: The major objectives of this research are:

(1) Construction of physical models of the respiratory tracts of man and several animal species;

(2) Measurement of the deposition of aerosols as small as 0.01 micron diameter in the models;

(3) Determination of deposition of selected particle sizes in animals and in man if practical;

(4) Development of a theoretical model of deposition in the lung based on the above results;

(5) Field evaluation of the physical-theoretical model.

MAJOR FINDINGS: Based on morphometric data collected in Stage I, a lobe by lobe anatomical model of the tracheobronchial tree in man and three experimental animals has been generated. The models can be presented as sets of numbers describing typical paths representative of the conducting airways from trachea to terminal bronchioles. Regional differences are evident, and these differences can be used to interpret deposition patterns seen in experimental animals.

The most obvious differences within species relate to average path lengths to different lobes. These have been used to help explain regional differences in aerosol deposition in rodent species. The path lengths for Beagle dog and man are comparable, for although the path lengths within lobes are shorter for dog, the trachea of the dog is much longer than the trachea of man.

The morphometry data is available as a laboratory report: "TB Geometry: Human, Dog, Rat, Hamster" LF 53, 1976, 600 pp.

The development of the physical models of the respiratory tract has progressed to the testing of various types of models of respiratory tract compartments.

The concept of using interchangeable modules for these model compartments allows their testing together, individually, or in any combination. An important concept developed in this work is the use of inter-module aerosol samples for determination of particle deposition in the various compartments rather than attempting to measure the actual deposition. This principle will greatly simplify field studies with physical models.

Deposition studies with hamsters and rats have been completed with monodisperse aerosols of ^{169}Yb-labeled aluminosilicate particles formed by heat fusion of clay aerosols containing ion-exchanges ^{169}Yb. The data has been completely analyzed and a report is available.

The inhalation exposure equipment for the beagle dog deposition studies and the longitudinal scanning equipment for measurement of regional deposition have been assembled and tested. The exposure system incorporates a new principle, concurrent flow spirometry, for maintenance of uniform aerosol concentrations and measurement of inspiratory and expiratory volumetric flow rates. Improved aerosol generation techniques for these experiments are in hand.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Improved respiratory tract deposition models are needed to develop insight into the manner in which the comparative morphology of the lung influences the deposition of inhaled particles in experimental animals and man. In particular, such a model would aid in establishing quantitative dose-response relationships, laboratory experiments and would effect more meaningful extrapolations to human exposure situations.

PROPOSED COURSE: This project has been amended to include the study of chemical-particulate aerosols.

PUBLICATIONS

Phalen, R.F., Yeh, H.C., Raabe, O.G. and Velasquez, D.J.: Casting the lungs in situ. Anatomical Record 177(2):255-263, 1973.

Yeh, H.C.: Use of heat transfer analogy for a mathematical model of respiratory tract deposition. Bull. Math. Biology 36:106-116, 1974.

Phalen, R.F., Yeh, H.C., Raabe, O.G., Hulbert, A.J. and Crain, C.R.: Comparative airway anatomy in the human, beagle dog, rat and hamster and implications in inhaled particles. Health Physics 27:6, 1974.

Phalen, R.F. and Raabe, O.G.: Aerosol particle size as a factor in pulmonary toxicity. Proceedings of the Fifth Annual Conference on Environmental Toxicology (In press).

Yeh, H.C., Hulbert, A.J., Phalen, R.F., Velasquez, D.J. and Harris, T.D.: A stereoradiographic technique and its application to the evaluation of lung costs. Investigative Radiology 10:351-357, 1975.

Yeh, H.C.: A note on the inertial depositon of particles in a bend. J. Aerosol Science (In press).

Raabe, O.G., and Yeh, H.C.: Principles for inhalation exposure systems using concurrent flow spirometry (In press).

Raabe, O.G., Yeh, H.C., Newton, G.H., Phalen, R.F. and Velasquez, D.J.: Deposition of inhaled monodisperse aerosols in small rodents (In press).

Phalen, R.F.: Respiratory tract morphology: Summary of a conference. Bioscience 24(10):612-614, 1974.

Phalen, R.F., Yeh, H.C., Raabe, O.G., Hulbert, A.J. and Crain, C.R.: Comparative airway anatomy in the human, beagle dog, rat and hamster and implications in inhaled particles. Health Physics 27(6):634, 1974 (Abstract).

Phalen, R.F., and Raabe, O.G.: Aerosol particle size as a factor in pulmonary toxicity (In press).

MEDICAL UNIVERSITY OF SOUTH CAROLINA - CHARLESTON, SC (NIH-NO1-ES-5-2152)

TITLE: "Characterization of Mouse Esterases with Acrylamide Gel Electrophoresis and Isoelectric Focusing"

CONTRACTOR'S PROJECT DIRECTOR: Robert C. Allen, Ph.D.

PROJECT OFFICER (NIEHS): Lawrence R. Valcovic, Ph.D.

DATE CONTRACT INITIATED: June 30, 1975

CURRENT ANNUAL LEVEL: $43,221

PROJECT DESCRIPTION

OBJECTIVES: The group of enzymes that exhibit esterase activity with non-specific substrates may represent as many as 20 genes in mice. However, starch gel electrophoresis is not sufficiently sensitive to resolve and identify all of the bands showing esterase activity. Acrylamide gel electrophoresis offers increased sensitivity and, when employed with a pH gradient (isoelectric focusing), provides the capability of identifying each band by its isoelectric point. This information can then be utilized to design buffer systems to amplify various regions with esterase activity and thus enable them to be readily incorporated into the current mutational assay system. An increase of 20 genes will greatly enhance the mutational sensitivity and effectively reduce the time and cost of testing.

METHODS EMPLOYED: Acrylamide gel electrophoresis and thin slab gel isoelectric focusing are used to characterize esterase patterns from various mouse tissues. Studies are carried out using DBA/2J and C57BL/6J inbred strains as well as the B6D2 interstrain hybrid. Various pH ranges of ampholines are used to provide maximal resolution of band with esterase activity.

MAJOR FINDINGS: The contractor has obtained reproducible pH gradients with isofocusing. The gradient is linear and 30 esterase activity bands can be visually differentiated. Isoelectric point determinations have been completed on kidney esterases from B6D2 animals; these are reproducible within 0.02 pH units.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The potential genetic risk to the human population from exposure to environmental chemicals cannot be estimated without adequate quantitative data in whole animal test systems. There is currently only one system available that is capable of providing this information, but it requires thousands to tens-of-thousands of animals to detect a response from moderate to weak mutagens. This project is one of several in a program designed to develop systems which utilize a large number of genes per individual to increase the sensitivity of detection. The practical use of these systems will be for quantitative risk estimation of presumed mutagens as well as establishing base-line data in laboratory animals for reference use with new procedures for monitoring the human population for genetic alterations.

PROPOSED COURSE: Efforts to determine the isoelectric points of esterases of DBA/2J and C57BL/6J will continue. Two dimensional electrophoresis will provide additional information on enzyme structure. The final phase of the program will be to determine the reliability of the procedure and to do a cost analysis to determine whether the technique is indeed practical for wide-scale use.

Michigan Department of Public Health
Bureau of Disease Control and Laboratory Science
Lansing, Michigan 48914

TITLE: " Human Health Consequences of Polybrominated Biphenyl Contamination of Farms in Michigan"

CONTRACTOR'S PROJECT DIRECTOR: Kenneth Wilcox, M.D.

PROJECT OFFICER: NIEHS - M. W. Anderson, Ph.D.
NCI - Gary Flamm, Ph.D.
CDC - Clark Heath, M.D.

DATE CONTRACT INITIATED: May, 1976

CURRENT ANNUAL LEVEL(NIEHS SHARE): $10,000

PROJECT DESCRIPTION

OBJECTIVES: This study is in its initial stages. We plan to obtain some disposition data on the subjects exposed to the polybrominated biphenyls (PBBs), i.e., blood levels and some tissue biopsies. Monitoring toxicological symptoms will be done for a long period of time (10-20 yrs). We are discussing possible laboratory experiments, such as pharmacokinetic studies in animal species, which would supplement the human studies.

METHODS EMPLOYED: To define and recruit a cohort of PBB-exposed individuals in Michigan and to arrange for their long-term periodic follow-up by the Michigan Department of Public Health. Blood samples and tissue specimens will be obtained periodically.

MAJOR FINDINGS: This contract is in its initial phase. Previous work by the Michigan Department of Public Health has shown that high blood levels of PBBs exist in some of the exposed individuals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A large group of individuals were exposed to these chemicals. They are similar to other persistent environmental contaminants such as the polychlorinated biphenyls. Thus, it is important to collect tissue disposition and toxicologic data for scientific reasons as well as for the health monitoring of the exposed individuals.

PROPOSED COURSE: Because of the suspected persistence of PBBs in the human body, long-term health monitoring of the exposed individuals is essential. Studies on the persistence of these compounds in the human body will also be done.

MIDWEST RESEARCH INSTITUTE, KANSAS CITY, MISSOURI (NIH-NIEHS-72-2090)

TITLE: "An Appraisal of Environmental Exposure to Heavy Metals"

CONTRACTOR'S PROJECT DIRECTOR: Ivan C. Smith, Ph.D.

PROJECT OFFICER (NIEHS): Warren T. Piver, Ph.D.

DATE CONTRACT INITIATED: April 17, 1972

CURRENT ANNUAL LEVEL: $55,000

PROJECT DESCRIPTION

OBJECTIVES: To explore environmental contamination with specific metals which may be hazardous to health. To search the literature and other reports and to seek expert opinions from industry in order to compile these data on potential hazards of specific metals. Special emphasis is placed on determination of the physical parameters and chemical properties, valency, reactivity, etc., as the metal compound makes contact with man. The information is to be put together in a document on each of the metals of specific interest.

MAJOR FINDINGS: Within the past year, a report on silver has been produced. These reports have a special significance because they attempt to define the chemical state of the metal in the environment as a result of its release due to intended use or as a contaminant in air and water. The form of the metal and the route of exposure have an important influence on its hazard potential. This type of information distinguishes these reports from the many other reports being produced on metals.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This surveillance is essential to stay abreast of changes in environmental contamination by certain industrial or communal practices involving different metals or different forms of metals, or their complexes. The information will determine whether research on the health effects of the compound in question should be undertaken and in which direction it should go.

PROPOSED COURSE: The information is being gathered for a number of metals of importance from the toxicological viewpoint. A report on zirconium is forthcoming. This will be followed by a report on thallium.

KEYWORD DESCRIPTORS: heavy metals; sources of environmental release; uses; distribution; metal toxicity.

MIDWEST RESEARCH LABORATORIES, KANSAS CITY, MISSOURI (NIEHS-72-2084)

TITLE: "Evaluation of the Environmental Toxicant, Vinyl Chloride Monomer"

CONTRACTOR'S PROJECT DIRECTOR: C. C. Lee, Ph.D.

PROJECT OFFICER (NIEHS): J. S. Woods, Ph.D.

DATE CONTRACT INITIATED: December 31, 1974

CURRENT ANNUAL LEVEL: $147,365

PROJECT DESCRIPTION

OBJECTIVES: This contract has been concerned with the toxic effects of the commercially available dithiocarbamate fungicides, ferbam and thiram, and their metabolic products. More recently, experiments have been designed to further determine the laboratory parameters associated with the toxicity of vinyl chloride and other monomers. The specific objectives of this new study can be summarized as follows: (1) to determine the biochemical and pathological alterations in specific organ functions of rats and mice chronically exposed to atmospheres containing vinyl chloride monomer (VCM) and (2) to establish and correlate response profiles of VCM for individual species with clinical data derived from epidemiological toxicity studies of VCM in laboratory animals with regard to more effectively predicting toxicity in humans.

METHODS EMPLOYED: Conventional chromatographic procedures, U.V., visible I.R. spectroscopy, histological techniques, radiotracer analyses, hematology, clinical blood chemistry, immunological tests, and cytogenic analyses.

MAJOR FINDINGS: Long-range toxicological effects of ferbam and thiram and acute toxic effects of maneb, zineb, ferbam, and thiram were studied in rats and mice. Toxic signs in rats following exposure to 1.2% ferbam in the diet for 13 weeks include decreased erythrocyte count, hematocrit and hemoglobin, increased reticulocytes, BUN, relative organ weights, and hemosiderin in the spleen and lymph nodes. Thiram was toxic at 0.05% in the diet for 6 weeks causing an increased percentage of neutorphils with a decreased percentage of lymphocytes, elevated BUN, increased severity of spontaneous lesions, increased relative organ weights, and, at lethal doses, tubular degeneration of the testicals with atypical spermatids in the epididymus.

Embryotoxicity was demonstrated when pregnant rats were administered high doses of thiram, but significant malformations were not observed. Chronic toxicity studies of ferbam and thiram are being conducted and data should be available in the near future.

Cytotoxicity studies indicated that the dithiocarbamate moiety of ferbam, thiram, and maneb was responsible for their toxicity to mammalian cells at the cellular level.

Vinyl chloride monomer and vinylidene chloride studies were initiated in September 1975. Rats and mice of both sexes are being exposed for 6 hours

per day, 5 days per week to 50, 250, or 1000 ppm vinyl chloride or to 50 ppm vinylidene chloride. Hematologic, pulmonary, immunologic, biochemical, and cytogenic analyses being conducted on exposed animal tissues have thus far indicated that no alterations from parameters measured in control animals have occurred. Continuing studies will evaluate the effects of vinyl chloride and vinylidene chloride exposure at 13, 26, 39, and 52 weeks, and for up to one year following cessation of exposure to these agents.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These investigations will establish the effect and nature of chronic toxicities of selected environmental chemicals using rats and mice. This information will serve to form a rational basis for evaluation of the potential human health hazards produced by the use and exposure of these chemicals.

PROPOSED COURSE: To continue the specified objectives of the contract in relationship to the assessment of chronic toxic effects of various environmental chemicals. This contract also provides the NIEHS with a "quick response" capability to study environmental chemicals that are thought to be potentially hazardous to human health.

KEYWORD DESCRIPTORS: long-range toxicological evaluation; metal-containing dithiocarbamate fungicides; environmental toxicants; vinyl chloride monomer; vinylidene chloride monomer; biochemical and pathological alterations in specific organ functions; chronic exposure; potential human health hazards.

PUBLICATIONS

Hodgson, J. R., Hoch, J. C., Castles, T. R., Helton, D. O., and Lee, C-C.: Metabolism and disposition of ferbam in the rat. Toxicol. Appl. Pharmacol. 33: 505-513, 1975.

Short, R. D., Russell, J. Q., Minor, J. L., and Lee, C-C.: Developmental toxicity of ferric dimethyldithiocarbamate (ferbam) and bis(dimethylthiocarbamoyl) disulfide (thiram) in rats and mice. Toxicol. Appl. Pharmacol. 33: 1975.

MILES LABORATORY - ELKHART, INDIANA (NIEHS-73-2113)

TITLE: "Genetic tests to characterize specific locus mutants in Neurospora crassa"

CONTRACTOR'S PROJECT DIRECTOR: J. L. Colbourn, Ph.D.

PROJECT OFFICER (NIEHS): Tong-man Ong, Ph.D., Senior Staff Fellow, Microbial Genetics Section

DATE CONTRACT INITIATED: April 9, 1973

CURRENT ANNUAL LEVEL: $65,945

PROJECT DESCRIPTION

OBJECTIVES: The primary objective of this contract is the genetic characterization of presumptive adenine-3 (ad-3) mutants of Neurospora crassa derived from forward-mutation experiments at the Institute and at Illinois State University (under contract with the Institute) with various environmental chemicals as well as various chemical carcinogens.

METHODS EMPLOYED: Stock cultures of presumptive ad-3 mutants have been characterized by a series of heterokaryon tests with various standard tester sets which determine the genotypes of the mutants, the complementation pattern of ad-3B mutants showing allelic complementation and also distinguishes point mutations from chromosome deletions. The contract also includes making mutants homokaryotic and preparing silica gel stock cultures.

MAJOR FINDINGS: During the past year more than 2000 mutants were made homokaryotic for adenine requirement and silica gel stock cultures were prepared. Mutants which have been successfully characterized include those induced by MNNG in haploid wild-type and in six different haploid UV sensitive strains and those induced by x-ray, various chemical carcinogens, nitrofurans and antischistosomal drugs in various two-component heterokaryons.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This contract relates to various programs in this Institute where it is essential to characterize the mutants induced at specific loci in this organism with regard to the type of lesion (e.g., point mutation or chromosomal deletion as well as the nature of the damage to the DNA at the molecular level). This is essential in our program to determine the correlation between carcinogenic and mutagenic activity as well as to determine whether chemical carcinogens characteristically produce any particular type of lesion at the molecular level. In addition, the characterization of the specific locus mutants produced by environmental chemicals enables us to better determine their relative hazard for man.

PROPOSED COURSE: Mutants which will be analyzed in the future include (1) carcinogen-induced mutants in excision-repair deficient strains, (2) carcinogen-induced mutants in standard wild-type strains, (3) radiation-induced mutants in two-component heterokaryons homozygous for excision-repair deficient strains (uvs-2 or upr-1) and (4) carcinogen-induced mutants in the latter strains as well as in heterokaryon 12.

NATIONAL MARINE FISHERIES SERVICE, BEAUFORD, NORTH CAROLINA
(Interagency Agreement Between NIEHS & NOAA)

TITLE: "The Physiological Effects of Arsenic, Cadmium, and Copper of Marine Shellfish"

CONTRACTOR'S PROJECT DIRECTOR: David W. Engel, Ph.D.

PROJECT OFFICER (NIEHS): B. A. Fowler, Ph.D.

DATE CONTRACT INITIATED: July 23, 1975

CURRENT ANNUAL LEVEL: $50,000

PROJECT DESCRIPTION

OBJECTIVES: This study was undertaken to determine the physiological, biochemical, and ultrastructural effects of arsenic, cadmium, and copper on tissues of marine shellfish in comparison with those previously observed in mammals. Large numbers of shellfish will be exposed to these elements for subsequent incorporation of freeze-dried shellfish meats into rat diets for evaluation of pharmacokinetic and toxicological differences in comparison to similar doses of these metals administered in drinking water.

METHODS EMPLOYED: Flowing seawater systems with continuous trace element injection, atomic absorption spectroscopy, radiotracer analyses, histological and ultrastructural techniques, cellular respiration studies.

MAJOR FINDINGS: At this early stage in the project, a suitable seawater system has been developed for dosing of oysters with cadmium and copper. Oysters have been exposed to cadmium and copper (.01 ppm) for 4 weeks and their uptake of these elements studied. Tissue concentrations of cadmium (410 ppm) and copper (360 ppm) were found in freeze-dried meats of these animals indicating a much faster and greater capacity for cellular accumulation of these elements than is known to exist in mammals. These freeze-dried tissues have been delivered to NIEHS for incorporation into rat diets. During the next year, glucose metabolism and oxygen consumption in similarly dosed oysters will be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: These studies will provide a basis for evaluating cellular responses to chronic low level trace metal exposure in tissues of relatively simple organisms in comparison to those observed in mammals.

Data generated from this effort should also prove useful in evaluating differences in trace metal pharmacokinetics and toxicity following ingestion of metal contaminated marine shellfish.

PROPOSED COURSE: To continue the specified objectives of this interagency agreement in relation to understanding the subcellular mechanisms of trace element uptake and toxicity in marine organisms in comparison to mammals. This arrangement also provides a mechanism for estimating potential human risk following ingestion of metal contaminated marine shellfish.

KEYWORD DESCRIPTORS: arsenic, cadmium, and copper uptake by oysters; comparative cellular mechanisms of trace metals uptake and toxicity; contaminated shellfish.

INFORMATION CENTER COMPLEX/INFORMATION DIVISION
OAK RIDGE NATIONAL LABORATORY - OAK RIDGE, TENNESSEE 37830
(Interagency Agreement Between NIEHS and ORNL)

TITLE: "Environmental Mutagen Information Center"

CONTRACTOR'S PROJECT DIRECTOR: Mr. John S. Wassom

PROJECT OFFICER (NIEHS): H. V. Malling, Ph.D.

COLLABORATING INSTITUTE: National Cancer Institute; Energy Research and Development Administration

DATE CONTRACT INITIATED: July 1, 1970

CURRENT ANNUAL LEVEL: $166,000

PROJECT DESCRIPTION

OBJECTIVES: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize and disseminate chemical mutagenesis information.

MAJOR FINDINGS: EMIC's data file at the end of March 1976 contained 16,300 bibliographic entries. Of these, approximately 14,000 have been indexed with respect to agent(s), organism(s), and Chemical Abstract Service Registry Number(s). The Center processes requests for information at a rate of 2-3 per day. These requests come primarily from governmental agencies in the United States or government supported research in universities. Several requests from other countries are also received. The EMIC data bank can now be screened by ToxLine.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Many compounds with widespread distribution in man's environment are known to be mutagenic in laboratory test organisms. Man's constant contact with mutagens may pose more danger to the genetic health of the human population than does radiation. The awareness of this problem among scientists, administrators and laymen created a need to make reference to published reports on tests for chemical mutagenesis readily available. This is important because these reports are scattered throughout a great number of publication sources (1550). EMIC has helped several national commissions in collecting and extracting the data from the literature. For the Institute, and especially the Environmental Mutagenesis Branch, EMIC saves more than one man-year of time for the personnel in the Branch by supplying their information needs.

PROPOSED COURSE: EMIC will continue to monitor the world scientific literature for reports on chemical mutagenesis. It will issue its annual literature survey in the middle of the fiscal year. This report will include an index to test organisms or test objectives as well as indexes for agents and selected title keywords. As great a portion as possible of EMIC's total bibliographic entries will be keyworded with respect to agent, test organism and test object. EMIC will continue to answer selected requests for special information from

the scientific, educational and industrial communities. Tabular extracts of the data from literature will be added to the data file as they are prepared in conjunction with the timely state-of-art reports on subjects of current scientific interest.

PUBLICATIONS

EMIC Staff: A survey of the 1974/75 Literature of Chemical Mutagenesis, ORNL-EMIC-7, 1976.

Shelby, Michael D.: Chemical Mutagenesis in Plants, ORNL-EMIC-8, 1976.

Malling, H.V. and Wassom, J.S.: The mutagenic action of environmental chemicals (in preparation for The Handbook of Teratology, to be published by Academic Press, New York, James Wilson and F. Clark Frazier, Editors).

Shelby, Michael D., de Serres, F.J. and Stine, Gerald J.: Ultraviolet inactivation of conidia from heterokaryons of Neurospora crassa containing UV-sensitive mutations. Mutation Research 27:45-58, 1975.

Shelby, Michael D., Stine, Gerald J. and de Serres, F.J.: Gamma-ray inactivation of conidia from heterokaryons of Neurospora crassa containing UV-sensitive mutations. Mutation Research (in press).

Von Halle, E.S., Generoso, W.M. and EMIC Staff: Chemical mutagenesis in mammals. A survey on the effects of chemicals on mammalian germ cells, ORNL-EMIC-6, 1976.

OAK RIDGE NATIONAL LABORATORY, U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION, OAK RIDGE, TENNESSEE (ERDA 40-524-75)

TITLE: "Environmental Teratology Information Center (ETIC)"

CONTRACTOR'S PROJECT DIRECTOR: John S. Wassom, B.Sc.

PROJECT OFFICER (NIEHS): Robert E. Staples, Ph.D.

DATE CONTRACT INITIATED: August 15, 1975

CURRENT ANNUAL LEVEL: $200,000

PROJECT DESCRIPTION

OBJECTIVE: To establish a teratology information center.

METHODS EMPLOYED: (1) To collect reprints of all publications that contain data pertinent to determination of the teratogenic potential of environmental agents or conditions to which man is exposed; (2) To use facilities available at Oak Ridge National Laboratories to enter extracts from the relevant references onto magnetic tape such that the information contained can be readily recalled for use; and (3) To encourage scientific evaluation of the adequacy of available data on high priority agents for determination of teratogenic potential.

MAJOR FINDINGS: Initial effort is concentrated upon accumulation of data from studies conducted on warm-blooded animals, whether the result is positive or negative. When this first priority block of information is well underway, it is intended that other blocks of information will be included, e.g., methodology, embryology, human syndrome identification, placental transfer, pharmacokinetics, metabolism studies.

The information is indexed by agent, ETIC access number, Chemical Abstract Services Registry Number, Wisswesser Linear Code, test animal, and investigator. Ultimately, about eighteen information fields are planned to be extracted from each reference; to date only the first six of these fields are being completed. A chemical dictionary will list all synonyms of agents studied and information pertinent to conducting tests for teratogenicity of each chemical.

Current studies are located by personal contact with teratologists, by manual scanning of about 30 key journals, and by automated scanning of available computer tapes from large multidisciplinary data bases (e.g., Chemical Abstracts) via a teratology profile. Earlier studies are located through use of teratology files made available by individuals and organizations.

Names and addresses of members of the American and European Teratology Societies have been entered; similar information from the Japanese Teratology Society will be available upon translation into English.

An advisory committee has been initiated to guide the development of ETIC.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Teratology data on chemical and other agents are widely scattered in the literature, and there is currently no central file listing agents which have been tested, let alone to what extent, or in what species. Teratology data are often included in publications on general toxicity and the chemicals tested often do not appear in titles, hence data are very difficult to locate. This information is necessary for those responsible for assigning priorities for study and for evaluating the teratogenic potential of individual agents. Creation of ETIC will permit ready access to the references containing teratology on each agent and will provide an extract of the pertinent information contained. Through use of ETIC, unplanned duplication of published studies can be prevented and it is planned that names of investigators with studies in progress on given agents will be listed.

PROPOSED COURSE: To continue information input on the first six fields, to select the optional computer system for handling extract data, to expand the advisory committee, to finalize the form and content of the next 12 fields, and to begin the complete extracting of pertinent articles.

KEYWORD DESCRIPTORS: teratology information center; warm-blooded animals; methodology; embryology; human syndrome identification; placental transfer; pharmacokinetics; metabolism studies.

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (UCLA)

TITLE: "Effects of 60 Hz Fields on the Mammalian Central Nervous System"

CONTRACTOR'S PROJECT DIRECTOR: W. Ross Adey, M.D.

PROJECT OFFICER (NIEHS): Donald I. McRee, Ph.D.

DATE CONTRACT INITIATED: October 1, 1975

CURRENT ANNUAL LEVEL: $99,994

PROJECT DESCRIPTION

OBJECTIVES: The objectives of this research are to determine the effects of 60 Hz fields from high voltage transmission lines on the central nervous system of mammals.

METHODS EMPLOYED: Environmental field conditions similar to those found in the vicinity of high voltage transmission lines will be simulated. These field gradients will range from 1 to 2000 volts/meter. The effects of these fields on the biological rhythms in rats and conditional behavior in monkeys will be evaluated. The effects on the neuroendocrine mechanism in rats and primates will be examined by using 17-hydroxysteriods, calcium, phosphorus, vanilmandelic acid and other urinary indicators of biorhythmicity. Changes in the neurochemistry of brain tissue of cats will be studied. These studies will include the measurement of both Ca and amino acid effluxes from the cerebral cortex of the cat.

MAJOR FINDINGS: The facilities for the exposure have been completed. The facilities allow exposure to sinusoidal 60 Hz fields of intensities up to 1000 volts/meter. The chambers for holding rats during irradiation have been constructed with light emitting diodes and fiber optics attached for monitoring movement. Preliminary studies using isolated chick brain has shown that the calcium flux with 60 Hz fields is three orders of magnitude less than at frequencies of 6-20 Hz.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The electric power industry is planning to construct high voltage transmission lines with voltages up to 1200 kV. In the vicinity of the lines, high electric and magnetic field gradients will be present. The health effects of these fields are presently unknown. This research will contribute to the determination of whether these fields do or do not constitute a health hazard. This project contributes to the mission of the Institute in evaluating the health effects of physical factors in the environment.

PROPOSED COURSE: The exposure of biological specimens to high voltage power line type fields will proceed. The specific studies to be accomplished include:

1. Effects on biological rhythms in rats,

2. Effects on conditional behavior in primates,
3. Effects on neuroendocrine mechanisms in rats and primates,
4. Effects on the neurochemistry of brain tissue.

UNIVERSITY OF MINNESOTA, DULUTH, MINNESOTA (NO1-ES-4-2149)

TITLE: "Collection and Storage of Biologic Specimens Exposed to Orally Ingested Asbestos Fibers"

CONTRACTOR'S PROJECT DIRECTOR: R. E. Carter, M.D.

PROJECT OFFICER (NIEHS): J. L. Braun, Ph.D.

DATE CONTRACT INITIATED: November 5, 1973

CURRENT ANNUAL LEVEL: $7,000

PROJECT DESCRIPTION

OBJECTIVES: Storage of specified biologic samples from human and non-human sources having varying exposures to oral ingestion of asbestos fibers.

METHODS EMPLOYED: Collected specimens will be identified and preserved to assure usefulness for future analyses.

MAJOR FINDINGS: The contractor has been successful in collection and storage of specimens as proposed.

SIGNIFICANCE TO BIO-MEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: This study will provide specimens for study of asbestos tissue burden information basic to determination of the health hazard potential of such exposure. Such studies are an integral part of the NIEHS efforts to determine the magnitude and significance of hazards inherent in long-term, low-level exposures to various environmental agents.

PROPOSED COURSE: This contract will be continued to provide for long-term retention of specimens collected under this contract.

KEY WORD DESCRIPTORS: Environment, health, epidemiology, asbestos

CANCER RESEARCH AND TREATMENT CENTER - THE UNIVERSITY OF NEW MEXICO
ALBUQUERQUE, NEW MEXICO (NO1-ES-5-2101-02)

TITLE: "Relationship Between Mortality and Ambient Air in the New York City Metropolitan Area"

CONTRACTOR'S PROJECT DIRECTOR: Robert W. Buechley, Ph.D.

PROJECT OFFICER (NIEHS): Michael D. Hogan, Ph.D.

DATE CONTRACT INITIATED: September 16, 1974

CURRENT ANNUAL LEVEL: $42,229

PROJECT DESCRIPTION

OBJECTIVES: The major objectives of this research are: (1) To analyze daily mortality data from the New York metropolitan area from the period 1967-1971 (or later, if possible) using previously developed methodology to assess the possible correlation between mortality and air pollutant concentrations, particularly sulfur oxides; and (2) To perform analyses to ascertain comparability between this later determination and the determination previously made by the principal investigator for the five year period from 1962-1966.

MAJOR FINDINGS: The principal investigator found that measured sulfur dioxide levels continued to correlate with daily deaths in the New York-New Jersey metropolitan area for the years 1968-1972. Since sulfur dioxide levels were markedly reduced over this time span, he concluded that dose-response relationships cannot be defended, and that even precursor status seems difficult to allow. However, it should be pointed out that the observed results are highly dependent on the particular format that is used to define various co-factors in the model, such as the annual cycle.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: With the emergence of the "energy crisis" and the potential increased use of fuel sources high in sulfur content, the significance of an investigation of the possible association between fluctuations in daily mortality ratios and measures of sulfur oxide levels is clear.

PROPOSED COURSE: This project has been terminated.

UNIVERSITY OF WASHINGTON - SEATTLE, WASHINGTON
(NIH-NIEHS-74-2151)

TITLE: "Detection of Point Mutations in Somatic Cells"

CONTRACTOR'S PROJECT DIRECTOR: Peter E. Nute, Ph.D.

PROJECT OFFICER (NIEHS): Heinrich V. Malling, Ph.D.

DATE CONTRACT INITIATED: January 1, 1974

CURRENT ANNUAL LEVEL: $88,000

PROJECT DESCRIPTION

OBJECTIVES: It is likely that an increase in the mutation rate in the human population would be detrimental, yet there is no system developed to monitor the human population for point mutations. The purpose of this investigation is to develop a simple system to measure point mutations in readily accessible human somatic cells. It is likely that the somatic cell mutation rate would reflect the germ cell mutation rate, and the rate of introduction of genetic defects into the human population.

METHODS EMPLOYED: Many different types of hemoglobin mutations exist in the human population. Some of these are point mutations in the α and β chain. Since such mutations occur in the germinal tissue, it is reasonable to assume that they also occur in the stem cells for the red blood cells (RBC). This type of mutation should result in an RBC which would contain an aberrant hemoglobin. By using a monospecific antibody to a number of aberrant hemoglobin types, such cells should be detected in samples from normal individuals after reaction of the antibodies with various fluorescent dyes. Antibodies are being produced against Hb-Wayne, Hb-Cranston, Hb-C and Hb-S.

MAJOR FINDINGS: Fluorescent monospecific antibodies to Hb-S have been produced. Reconstruction experiments have shown that by use of anti Hb-S one Hb-S cell can be detected among 10^6 normal cells. In normal human the frequency of Hb-S containing cells range at approximately 5×10^{-8}, which is low enough to allow for a positive detection of mutagenic effects.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A system able to detect mutations in the genome of readily accessible somatic cells would allow estimation of the mutation rate in the human population. It would allow investigation of individuals and populations exposed to a mutagenic risk. There is no simple technique for screening at present. The antisera would also be useful in investigation of thalassemia and related genetic hemoglobin disorders.

PROPOSED COURSE: When the technique is available, it will be used to investigate somatic cell mutation rates with respect to age and to investigate populations at mutagenic risk such as patients receiving cancer chemotherapy and those exposed to radiation. Screening programs would be able to identify populations exposed to mutagenic substances.

Virginia Commonwealth University
Richmond, Virginia
NO1-ES-6-2117

TITLE: "A Study of the Pharmacokinetics and Toxicity of Kepone in Humans and Laboratory Animals"

CONTRACTOR'S PROJECT DIRECTOR: Philip S. Guzelian, M.D.

PROJECT OFFICER (NIEHS): H. B. Matthews, Ph.D.

DATE CONTRACT INITIATED: December 15, 1975

CURRENT ANNUAL LEVEL: $78,801

PROJECT DESCRIPTION

OBJECTIVES: The recent discovery of accidental chronic exposure of a small community to Kepone® has presented many problems relating to immediate toxicity and unknown long term consequences. The goal of this proposal is to conduct a rapid, orderly, and systematic investigation of the absorption, distribution, biotransformation, and excretion of Kepone® in humans and animals to aid in understanding its toxicity and to provide a basis for rational therapy.

METHODS EMPLOYED: 1) Human Investigations. Initial clinical findings will be expanded to include tabulation of complete historical, epidemiologic, and physical findings in 25-30 symptomatic patients. Routine clinical lab tests will be done. Blood, saliva, and excreta will be analyzed for Kepone and metabolites. 2) Analytic Methods. Various types of solvent extractions will be used to remove Kepone and metabolites from biological samples, followed by TLC and GLC analysis. 3) Lab Animal Investigations. The relative rates and extent of Kepone excretion in urine, bile, and feces will be compared in different species by the usual methodologies.

MAJOR FINDINGS: The first reports are not yet in.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: A recent discovery of accidental industrial poisoning of workers with the pesticide Kepone and the possibility of contamination of the air, water, and sewage of a small Virginia community with this chlorinated hydrocarbon has created a need to rapidly develop biologic information regarding this agent. The proposed interdisciplinary studies will investigate the toxic and pharmacokinetic properties of Kepone using a combined approach of *in vivo* and *in vitro* systems including man. A well-conducted analysis of this problem may not only yield valuable information on Kepone but may set up a protocol for the investigation of similar incidents of environmental contamination which may occur in the future with other compounds.

PROPOSED COURSE: The contractor proposes to characterize Kepone-associated toxicity and to define Kepone pharmacokinetics in man and laboratory animals by an interdisciplinary approach which can conveniently be represented in three categories:

A. Human Investigations

1. by defining the clinical syndrome of Kepone-associated toxicity
2. by quantitative determination of the routes of Kepone excretion including analysis of enterohepatic recirculation of Kepone
3. by searching for products of Kepone biotransformation
4. by devising and cautiously evaluating treatment regimens

B. Analytical Methods Investigations

1. by defining optimal conditions for Kepone extraction and quantitation of biological samples from humans or animals
2. by defining optimal conditions for detecting and, if present, characterizing products of kepone biotransformation
3. by *in vitro* analysis of the binding of Kepone by various sorbents

C. Laboratory Animal Investigations

1. by delineating the pharmacokinetics of Kepone
2. by studying Kepone biotransformation *in vivo* and *in vitro*
3. by evaluating the effects of drug interaction on the parameters listed above (C1, C2)
4. by determining the effect of Kepone on hepatic microsomal function

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